terior) or away from (posterior) the source. The rate of recovery from hypothermia in animals receiving posterior exposure was significantly more rapid than either anteriorally exposed or sham-exposed animals.

When ethanol is administered to test animals, it results in hypothermia. Some studies have demonstrated that 2.45-GHz microwaves can attenuate ethanol-induced hypothermia (Lai et al. 1984b; Hjeresen, Francendese, and O'Donnell 1988). Also, permeability of the BBB to Evans blue dye is reduced in rats exposed to high-level MW and ethanol. In this experiment the left hemisphere of the brain was irradiated. Dye staining was observed only in that hemisphere and other normally leaky areas. The intensity of the stain was inversely related to the ethanol concentration (Neilly and Lin 1986).

Hjeresen, Francendese, and O'Donnell (1989) designed an experiment to see if MW-induced effects on ethanol hypothermia were associated with norandrenergic (NE) neurotransmitter systems. Neonatal rats received injections of the neurotoxin, 6-hydroxydopamine (6-OHDA), to produce lesions in norandrenergic neurons. NE levels in the cerebral cortex were about 10 times lower in controls versus 6-OHDA-treated animals. Animals exposed to both MW and 6-OHDA did not exhibit the marked reduction in ethanol-induced hypothermia characteristic of control animals exposed to MW but not 6-OHDA: "The results... suggest that microwave irradiation may, by an unexplained mechanism, act in a manner similar to norandrenergic β-antagonists" (Hjeresen, Francendese, and O'Donnell 1989). However, this conclusion has been disputed by Klauenberg and Merritt (1991), who interpret the data as "indicating that MW does not appear to interact with any of the NE pharmacological challenges. The most parsimonious interpretation is that it is premature to conclude that the NE system is involved in the effects of microwaves on EtOH-induced hypothermia." In a reply to this critique, Hjeresen (1991) reaffirmed the original interpretation.

Behavioral changes have been used as an end point in studies of combined effects. Subsequent to operant conditioning, monkeys were injected with fenfluramine, a serotonin depleter, then exposed at 2450 MHz while restrained. Behavior disruption was observed from the combined exposure (Galloway and Wazler 1977). In another study, escape-avoidance behavior in a small number of mice was examined. A stable baseline of behavior was established with exposure to 2450-MHz MWs at an average SAR of 45 W/kg. Animals were then treated with different doses of chlordiazepoxide, d-amphetamine, and chlorpromazine and microwaves. Animals treated with chlordiazepoxide and MW exhibited distinct changes in escape-avoidance behavior compared with exposure to MW alone (Monahan and Henton 1979).

A study using magnetic-resonance imaging conditions found that reductions of morphine-induced analgesia were greater for time-varying magnetic field than for RF fields. Static-magnetic fields did reduce morphine-induced analgesia, but not significantly (Prato et al. 1987).

In summary, research has focused on combined interaction between psychoactive drugs and microwaves, primarily at 2.45 GHz. Drugs were administered at doses that by themselves produce measurable effects in the test animals. The effects of some drugs were enhanced, while others were attenuated by acute exposure to microwaves (Lai 1992). It is not known if subtle effects in humans are possible from low doses of drugs, solvents, or other neuroactive substances found in the workplace. Patients under anesthesia or medication in health care facilities who receive therapeutic RF irradiation are a more likely population for these effects.

3.3.4.6 Behavior

Evaluation of behavior is a way to measure the health of the central nervous system (CNS) and associated systems. Behavioral effects are cited as the limiting condition in some exposure guidelines. For example, ANSI (1982) and IEEE (1992a) employ thresholds of reversible behavior disruption in test animals in establishing human exposure criteria, because behavioral effects were found to be the most sensitive effects that were understood.

Behavior is innate or learned. Innate or natural behavior, such as locomotion, eating, and reproduction, is inherent to an animal species. Effects on natural behavior are often evaluated in open-field tests with rodents. Simply, this involves a box with a grid on the floor. Typically, researchers observe behavior such as the animals' exploratory activity (ambulation over a number of squares) and vertical activity (rising onto hind legs) in the box.

There are two types of learned, or acquired, behavior. These are called respondents and operants and are determined by the type of response that is elicited by a stimulus. If the animal's response involves motor activity subsequent to stimulation, the response is called a respondent. In this case the introduction of a stimulus directly invokes a response. The aversion response to a bright light is a respondent involving motor activity, e.g., blinking. Operant responses are elicited when an animal is conditioned by a positive or negative stimulus, called reinforcement. For example, an animal may be conditioned to expect an audible tone immediately before receiving reinforcement. After conditioning the tone will serve as the stimulus to a motor response. Hence, the animal's behavior is modified by its response. Other examples include bar or lever pressing to receive food.

Studies have been performed mostly with rats but also with mice, chickens, dogs, and monkeys. Generally, exposures have been to pulsed or CW, 2.45-GHz microwaves. Most research on behavioral effects associated with RF radiation has utilized operant conditioning. Behavioral end points that have been evaluated include convulsions, work stoppage, work perturbation, endurance, perception of RF fields, and aversion (Justesen 1979). Results of a number of behavioral experiments are in Table 3-7, and literature reviews are available (Servantie and Gillard 1983; Elder and Cahill 1984; CDRH 1985; NCRP 1986; Blackwell and Saunders 1986; Heynick 1987; D'Andrea and de Lorge 1990; D'Andrea 1991).

Because this vein of experimentation is important in setting exposure criteria, the design of a few of the following studies will be discussed in some detail. First, however, it is important to place behavioral effects in a proper perspective, which O'Connor (1988) has done quite well:

> The fact that positive behavioral effects appear well below levels where other effects are reliably observed is due to the very nature of the system under investigation. The nervous system has evolved to be the first to respond to many environmental changes, and behavior thus often represents the body's initial warning signal. For this reason alone, the study of behavior will probably produce more false positives than the study of other systems. It is important to remember that behavior can be, but is not always, indicative of nervous system disturbance. Conversely, and of equal importance, is the fact that nervous system disturbance is not always biologically or behaviorally significant to the organism.

Five rhesus monkeys were exposed to various power densities (Table 3-7) for either 30, 60, or 120 minutes. Colonic temperatures were monitored during some experimental runs. Sitting monkeys were restrained in a styrofoam chair facing the MW source. The animals were trained in an operant task to receive food pellets. Upon depressing a lever in front of the right arm, either a low-frequency or a high-frequency audible tone was emitted. The low-frequency tone lasted for 0.5 seconds, and indicated no food was available. The high-frequency tone remained on until the monkey depressed a lever in front of the left arm, an action that also delivered a food pellet. Food pellets were not always available but were provided at variable intervals within some given time limits, called a variable interval schedule. If the left lever was depressed when food was unavailable (no high-frequency tone), a 0.5-second low-frequency tone was emitted. Animals were trained in this task for 70 sessions prior to actual exposure. Measures evaluated included the rate on the right and left levers, and detection response rate. Consistent

Table 3-7. Behavioral Studies

Species	Frequency (MHz)	SAR (W/kg)	Average Power Density (mW/cm^2)	Duration (d×min)	Effects	Reference(s)
			CW Exposures			
Monkeys (male)	2450 AM: 120 Hz	0.32 to 5.8	4 to 72	1×60	Response rate of lever presses decreased at highest dose rate; elevated colonic temperature	de Lorge 1976
Albino rats	2450 AM: 120 Hz	5.8	28	1×60	Threshold of behavior disruption of an operant task for 3 species	de Lorge 1978
Squirrel monkeys		2.5 or 4.5[a]	45	1×60		
Rhesus monkeys (male)		4.7	67	1×60		
Monkeys (male)	225 CW	3.2	8.1	1×60	Threshold of behavior disruption of an operant task	de Lorge 1984
	1300 Pulsed	4.5	57	1×60		
	5800 Pulsed	8.4	140	1×60		
Rats (male)	400, 500, 600, 700 CW	7, 11, 16, 14[b]	20	1×55	Shortest time to work stoppage at 600 MHz; time to work stoppage varies inversely with exposure level	D'Andrea, Gandhi, and Lords 1977
	600 CW	4, 6, 8, 16	5, 7.5, 10, 20	1×55		
Rats (female)	2450 CW	2.3	NR	110×300	Significant differences in one innate and one operant test; no difference in one avoidance behavior test	Mitchell, Switzer, and Bronaugh 1977

Table 3-7. (Continued)

Species	Frequency (MHz)	SAR (W/kg)	Average Power Density (mW/cm^2)	Duration (d×min)	Effects	Reference(s)
Rats (male)	2450 CW	1.23	0.5	80×480	Significant difference in stablimetric activity	D'Andrea et al. 1979
Rats (male)	2450 CW	0.14	0.5	90×420	Differences in 2/4 behavior measures; increased lever pressing in exposed animals	D'Andrea et al. 1986a
Rats (male)	2450 CW	0.14	0.5	90×420	Differences in 1/4 behavior measures: decreased lever pressing in exposed animals	DeWitt et al. 1987
Rats (male)	2450 CW	0.70[c]	2.5	98×420	Differences in foot shock response and shuttlebox avoidance test	D'Andrea et al. 1986b
Rats (male)	2450 CW	2.7[c]	10	1×420	Significant differences in activity and acoustic startle response	Mitchell et al. 1988
Rats (male)	2450 CW	2.7[c]	10	1×420	Difference in passive avoidance measures in U.S. test, not seen in eastern European results	Mitchell et al. 1989

Table 3-7. (Continued)

Species	Frequency (MHz)	SAR (W/kg)	Average Power Density (mW/cm^2)	Duration (d×min)	Effects	Reference(s)
Rats (male)	2450 CW	1.2, 1.8, 4.8	5, 7.5, 20	1×30	Increased DRL response rate; decreased FR response rate	Thomas et al. 1975
Rats	915 CW	7, 10, 17	NR	1×10	No effect on acquired taste aversion	Monahan and Henton 1977
PULSED EXPOSURES (UNLESS OTHERWISE NOTED)						
Rats (female)	1200 CW	2.4[c,d]	2.4	4×30	No significant differences	Frey, Feld, and Frey 1975
	1200	0.2[d]	0.2	4×30	Significant differences in shuttlebox performance	
Rats (male)	2450	6.3	NR	1×30	Initial decrease in exploratory activity;	Hunt, King, and Phillips 1975
		6.3, 11	NR	1×30	reduced swim speed late in the test; prompt, gross reduction in performance of 11-W/kg group; poor initial performance in discrimination task for both levels of SAR	
Rats (male)	1280	0.25 ±0.01	≤1, 5.5, 9.5, 10, 15	62×40	Threshold of behavior at SAR = 3.75 W/kg; 4.94 W/kg	de Lorge and Ezell 1980
	5620	0.19 ±0.003	7.5, 11.5, 16, 26, 31.5, 42, 48.5	183×40		

Table 3-7. (Continued)

Species	Frequency (MHz)	SAR (W/kg)	Average Power Density (mW/cm^2)	Duration (d×min)	Effects	Reference(s)
Rats (male)	600	0.4, 4	0.51, 5.1 average	1×55	No effect on work stoppage	D'Andrea, Gandhi, and Lords 1977
Rats (male)	1250	0.84, 2.5, 7.6, 23[e]	NR	4×10	Work stoppage at highest SAR	Akyel et al. 1991
Monkeys (male)	1300	0.05 to 0.8[c,f]	0.09 to 1.48 average	5×60	No significant differences in food-reinforced tasks	D'Andrea, Cobb, and de Lorge 1989
Rats (male)	3000	0.072[c], 0.057 to 0.087 in brain	See text	—	Significant effects on cognitive abilities	Raslear et al. 1993
Rats (male)	2450	0.15 to 0.4	0.5	750×1260	No effects on open field behavior	Johnson et al. 1983
Rats (female)	1300	1.5, 3.6, 6.7	NR	30×180 or 45×100	Clear differences at high dose rate	Lebovitz 1981
Rats (male)	2860	1.2, 2.4, 3.6, 4.8	5, 10, 15, 20	1×30	Variable responses on DRL and FR schedules	Thomas et al. 1975
	9600	0.5, 1.0, 2.0, 3.0	2.5, 5, 10, 15	1×30		
Rats (male)	2800	0.7, 1.7	5, 10	1×30	Significant differences in response to acquisition task at higher dose rate	Schrot, Thomas, and Banvard 1980

Table 3-7. (Continued)

Species	Frequency (MHz)	SAR (W/kg)	Average Power Density (mW/cm^2)	Duration (d×min)	Effects	Reference(s)
BEHAVIORAL TERATOLOGY						
Mice (female)	2450 Pulsed	38±3	NR	1×10	No differences in performance in a swimming maze	Chernovetz et al. 1975
Monkeys (female)	2450 Pulsed	0.0034, 0.34, 3.4	NR	10×5	No differences in locomotor behavior, significantly delayed time to reach dams for pups exposed at highest SAR	Kaplan et al. 1982
Rats (female)	2450 CW	16.5 to 5.5[g]	10	See text	Effect seen on swimming endurance and startle responsiveness	Galvin et al. 1986

[a]Sitting position: lower SAR with back straight, higher SAR curled over (typical position).
[b]SARs are estimates from prolate spheroidal models of medium rats. (Durney, Massoudi, and Iskander 1986). Exposures were made with the E-field vector parallel with the long axis of the rodent bodies.
[c]Whole-body average.
[d]SAR estimate from Elder and Cahill 1984.
[e]SARs in rat carcasses demonstrated that the spatial peak SAR was highest in the thoracic region, lowest near the pelvis, and intermediate in the abdomen. All measurements in the head region were within the noise level of the fiber-optic temperature sensors. Animals received a single exposure at each SAR with their bodies oriented parallel with the E field.
[f]The propagation vector was parallel with the body, and the E-field vector oscillated ear to ear. Each animal received a single, 60-minute exposure at a PRF of 2, 4, 8, 16, and 32 Hz. Peak power densities in all cases were 131.80 W/cm^2. WBA and local SARs were as high as, respectively, 0.08 and 1.44 W/kg at 32 Hz, while peak SARs were 8.3 W/kg for the whole body and 15.0 W/kg in the head.
[g]Fetal SARs were around 2 to 4 W/kg, while postnatal SARs decreased from 16.5 W/kg (day 2) to 5.5 W/kg (day 20) as the pups grew.
NR, not reported; AM, amplitude modulated; CW, continuous wave; FR, fixed ratio; DRL, differential reinforcement of low rate.

effects on behavior were only seen at the highest exposure, 5.8 W/kg and 72 mW/cm^2 for 60 minutes, as the monkeys became agitated, then took naps and slept. Approximately 10 minutes after exposure ceased, the animals became active again. Colonic temperatures displayed a logarithmic relationship with power densities greater than 16 mW/cm^2 as shown in Fig. 3-3. De Lorge (1976) observed that the animals adapted to MW exposure both in behavioral responses and in temperature measures. He suggests that the threshold for behavioral effects in rhesus monkeys exposed as described is between 50 and 70 mW/cm^2. In a later analysis, De Lorge (1984) proposed that the findings could be explained by the formation of a hot spot behind the center of the brain.

De Lorge (1978) studied behavior disruption of an operant task in albino rats, squirrel monkeys, and rhesus monkeys. Power densities were 0 to 75 mW/cm^2. Monkeys were restrained as discussed earlier, while rats were unrestrained. Animals were trained in a food-reinforced operant task. Behavior was disrupted at a lower-power density for rats than for monkeys, probably because the wavelength is closer to the resonant-absorption frequency

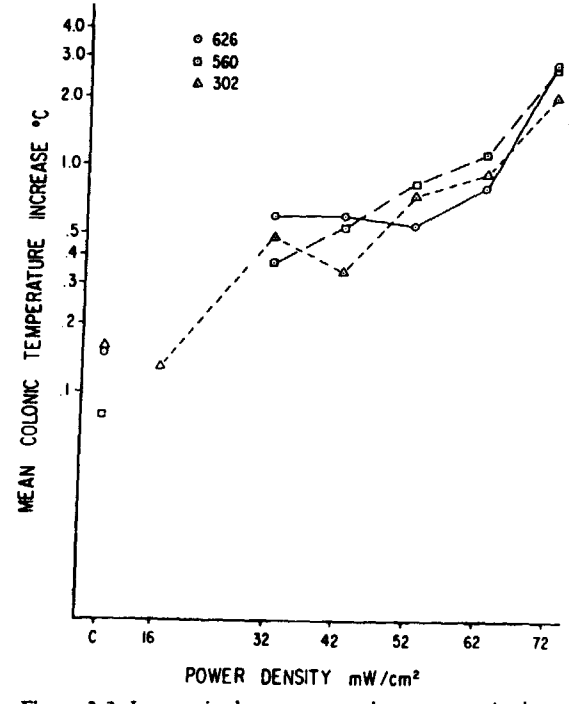

Figure 3-3. Increase in the average rectal temperature in three rhesus monkeys as a function of the incident power density. The numbers in the upper left refer to individual animals. From de Lorge (1976).

for rats. Thresholds of disruption are shown in Table 3-7. To disrupt behavior, de Lorge found it necessary to elevate body temperature more than 1 °C above control temperatures.

The importance of elevated body temperature was observed in an experiment where five rhesus monkeys were trained in a food-reinforced task, then exposed near whole-body resonance (225 MHz), above resonance (1300 MHz), and well above resonance (5800 GHz) for the species. Thresholds of disruption of the observing response are in Table 3-7. For comparison, at 2450 MHz, behavior disruption occurs at 4.7 W/kg (67 mW/cm^2). In all cases, behavior disruption was associated with increases in colonic temperature of around 1°C. At 225 MHz there was greater energy absorption per watt, and less absorbed energy was necessary to raise colonic temperature by 1°C. These data were used to analyze the ability of the SAR and power density to predict the observed effects: "By all accounts SAR is obviously a better predictor of response disruption than power density, but in both cases one has to take frequency into consideration. A more reliable, single index of behavioral disruption is a ΔT of colonic temperature of ~ 1°C" (de Lorge 1984). The change of temperature above baseline (average = 38.6° C) found by de Lorge is shown in Fig. 3-4. The ΔT increases exponentially for 225 MHz and logarithmically for 1.3 and 5.8 GHz as a function of power density (de Lorge 1984).

D'Andrea, Gandhi, and Lords (1977) examined the effect of frequency on operant behavior in five rats exposed at four different frequencies. In a second experiment, six rats

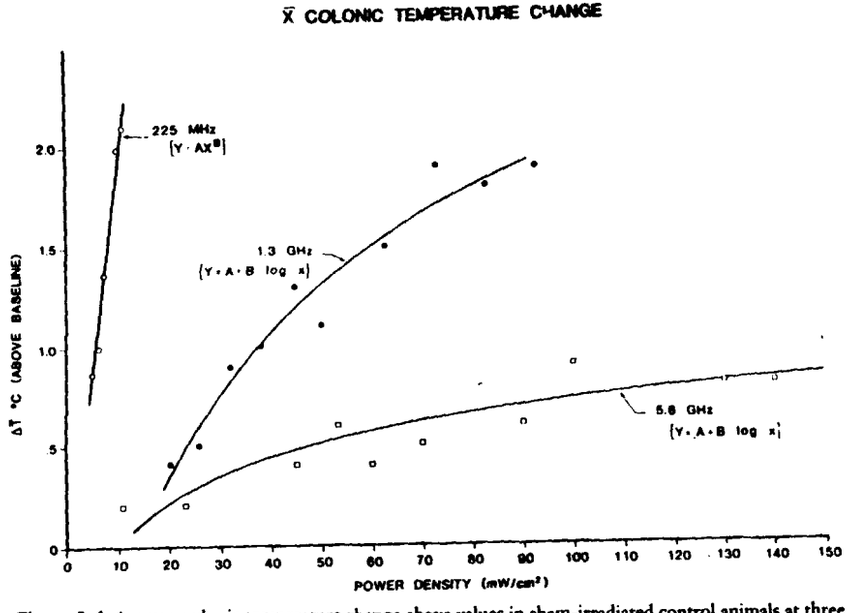

Figure 3-4. Average colonic temperature change above values in sham-irradiated control animals at three frequencies as a function of incident power density. From De Lorge (1984); used with permission.

were exposed at four different SARs at 600 MHz, as shown in Table 3-7. Animals served as their own controls, with baseline values determined prior to exposure. Colonic temperature was measured at the beginning and end of the treatment. Animals were trained to press a lever to receive food pellet reinforcement (work) and were evaluated on the time to work stoppage after beginning exposure. Work stoppage came most quickly and body temperature was highest when animals were exposed at 600 MHz, near resonance for the species. Frequency-dependent differences in time to work stoppage were statistically significant. The researchers observed all animals exposed at 20 mW/cm^2 licking their fur, which they speculate was to produce evaporative cooling. Work stoppage did not occur at the two lowest SARs at 600 MHz. At 8 W/kg work stoppage occurred after about 45 minutes and around 25 minutes at 16 W/kg. Colonic temperatures were highest at 16 W/kg. When the SAR was halved, the core temperature increase was reduced proportionately. At the lower SARs, colonic temperatures were slightly above baseline values.

Mitchell, Switzer, and Bronaugh (1977) found that average locomotor activity was significantly increased in MW-exposed rats, and nonreinforced food responses were markedly different. An ancillary observation was that four of the exposed animals lost fur on their backs, while this was not observed in control animals. There were "no striking abnormalities in behavior" between these animals and the nondepilated rats. Blackwell and Saunders (1986) questioned the observed depilation: "Whether this was due to some other cause is not clear, but it is possible that this was not due to the microwave exposure, and that the same cause might be responsible for the behavioural effects as well. So this result should be treated with caution."

D'Andrea et al. (1979) observed that activity on a stabilimetric platform was significantly decreased in MW-exposed animals. However, activity as measured by wheel running was not significantly affected. The difference in the outcome of these two measures was interpreted as providing evidence of thermal stress during and immediately following exposure, and recovery from thermal stress some time after exposure, since the tests were performed at different times following exposure.

D'Andrea et al. (1986a) encountered difficulties in the interpretation of results when no differences were seen to shock sensitivity or open-field performance, but significant differences were noted in the variability of the pooled data for the shuttlebox test and in the average values in the schedule-controlled behavior test. This may be due to individual responses and not to MW influence on the entire group of animals, because the observed variability was due to an inconsistent response within the exposed group. In the schedule-performance test, the exposed animals pressed the lever more than sham controls, but in a later study the exposed rats pressed the lever fewer times (DeWitt et al. 1987). "The disparate results of our two studies at 0.5 mW/cm^2 are not surprising since contradictory results should be expected at threshold levels of treatments" (D'Andrea et al. 1986a).

A number of researchers have examined the effects of pulsed RF radiation on behavior. Frey, Feld, and Frey (1975) saw no differences in preference to the radiated or nonradiated side of a shuttlebox during the first 2 days of exposure. For the last 2 days, the pulsed-exposure group (width = 0.5 ms, PRF = 1000 Hz) showed a moderate preference for the nonirradiated side of the shuttlebox.

As with CW studies, the body temperature is an important determinant in effects. Adult Wistar rats, exposed (6.3 W/kg, width = 2.5 ms, PRF = 120 Hz) such that body temperature was elevated by about 1.7°C, demonstrated a transient reduction in exploratory activity. In a swimming performance test, animals were tested in 24°C water immediately after exposure. Colonic temperatures in the 11 = W/kg group were ≥ 41°C, demonstrating severe hyperthermia. All exposed rats displayed a reduction in swimming speed, after swimming for some time. Animals exposed at the higher SAR showed "an observably gross impairment in performance for a few initial traverses, which was followed by a period of

apparent recovery to the controls' level of proficiency." After swimming around 100 traverses, their performance again declined. The authors credit the decrease in swimming speed late in the test to early fatigue (Hunt, King, and Phillips 1975).

De Lorge and Ezell (1980) observed resonant effects with pulsed exposures (1.28 GHz: width = 3 μs, PRF = 370 Hz; 5.62 GHz: width = 0.5 or 2 μs, PRF = 662 Hz) of Long-Evans rats. The threshold of behavior disruption was lower at 1.28 GHz than at 5.62 GHz, and evaluation of local SARs showed that the pattern of absorption was different for the two frequencies, with deeper penetration at the lower frequency (de Lorge and Ezell 1980). De Lorge (1984) noted no differences in effects due to pulse parameters (1.3 GHz: width = 3 μs, PRF = 370 Hz; 5.8 GHz: width = 0.5 or 2 μs, PRF = 662 Hz), but differences in disruption thresholds found among CW 225-MHz radiation and pulsed emissions were attributed to geometrical resonance.

If other physical parameters are held constant, the energy dose varies with the PRF, as shown in a study with Wistar rats exposed at a peak power of 1 megawatt (width = 10 μs), while the PRF was varied to produce different SARs as shown in Table 3-7. Prior to exposure, rats were trained in food-reinforced operant tasks with different schedules of reinforcement. At the highest SAR, which produced a peak SA of 14 kJ/kg, an absolute work stoppage was observed. Work stoppage was not observed at the other SARs. Colonic temperatures at the highest exposure were elevated by about 2.5°C. Colonic temperatures were also elevated by 0.7°C at 7.6 W/kg, but behavioral measures were not changed (Akyel et al. 1991). The findings are consistent with the hypothesis that behavioral effects have a thermal derivative.

D'Andrea, Cobb, and de Lorge (1989) delivered 3-μs pulses to confined rhesus monkeys at a number of PRFs, which doubled the SAR for each doubling of the PRF. Animals underwent operant conditioning for a complex multiple-schedule performance of food-reinforced tasks. No statistically significant differences were found for any measure. Other researchers exposed rats at peak powers in excess of 700 megawatts (3000 MHz; width = 80 ns; PRF = 0.125 Hz). This produced effects on some responses dealing with the processing of sensory information (discriminability, session time, and trial completions). This was interpreted as affecting cognitive function at levels less than the 0.4 W/kg value of WBA-SAR recommended in the safety standards. Exposure occurred at levels that would produce microwave hearing in the test animals, but testing followed exposure. Hence, the authors conclude that the observed effects are not associated with the hearing phenomenon (Raslear et al. 1993).

Long-term experiments have also evaluated behavioral end points, as mentioned in Section 3.3.1. Performance was not reliably affected in 14 open-field assessments spanning a 2-year period in rats exposed at an average SAR of around 0.15 to 0.4 W/kg (Johnson et al. 1983; Guy et al. 1985). Lebovitz (1981) exposed rats in individual waveguide exposure chambers (width = 1 μs, PRF = 600 Hz). No significant differences in operant behavior were observed at 1.5 W/kg, while measures at 3.6 were not reliably affected. At 6.7 W/kg, there were changes in visually cued and nonvisually cued responses. Lebovitz suggests that 3.6 W/kg may be around the threshold for the observed effects.

It has been suggested, as noted earlier, that some behavioral effects observed with pulsed microwaves may be due to MW hearing. For example, Thomas et al. (1975) exposed rats as shown in Table 3-7 (width = 1 μs, PRF = 500 Hz), finding highly variable results and no clear trend in response behavior (Thomas et al. 1975). The suggestion that the outcome may be due to auditory effects gains support because peak SARs, 200 to 8000 W/kg, are high enough to produce microwave hearing (Blackwell and Saunders 1986). It is possible that microwave-induced auditory effects (peak SARs estimated at 1.7 kW/kg) could explain the behavioral changes in a study by Schrot, Thomas, and Banvard (1980). Blackwell and Saunders (1986) hypothesize that the auditory effects could have "interfered with the discrimination of tone cueing" that was used in this study.

In the area of behavioral teratology, Chernovetz et al. (1975) exposed pregnant C3H-HeJ mice on day 14 of gestation, then allowed them to present naturally. There were no differences in MW-treated, mature offspring and controls in a swimming maze.

Kaplan and colleagues (1982) exposed pregnant squirrel monkeys and offspring (up to 6 months postpartum; see Section 3.3.4.2). They observed a significant difference for the high-dose-rate group in one of five perceptual-motor development tests, directed locomotion.

Galvin et al. (1986) divided offspring from MW-exposed and sham-exposed groups into two groups. One was exposed, while the other received no postnatal exposure or sham exposure. Motor activity, limb grip strength, negative geotaxis, and reaction time to an adverse thermal stimulus were not reliably affected. Startle responsiveness in exposed female pups was significantly elevated in both the experiment and a replicate. In the experiment, swimming endurance was significantly different at age 30 days for both prenatally exposed males and pre- and postnatally exposed males and females. By 100 days of age, there were no significant differences, and MW-exposed males exhibited greater swimming endurance than shams. In the replicate, all exposed groups had significantly decreased swimming endurance at 30 days (Galvin et al. 1986).

In summary, most behavioral studies have been performed with rats at MW frequencies, primarily 2450 MHz. MW irradiation has been shown to disrupt learned behavior, with specific changes in operant behaviors. A change in operant behavior observed frequently is reduction in the response rate (de Lorge 1983, 1985). For CW exposures, changes in two different end points were reported at SARs as low as 1.2 W/kg at 2450 MHz, although these data were highly variable (Thomas et al. 1975). Increased lever pressing was observed in an experiment at 2450 MHz with male rats exposed at 0.14 W/kg (D'Andrea et al. 1986a), while decreased lever pressing was found in another experiment (DeWitt et al. 1987). Other researchers did not detect the same outcome in two experiments with male rats exposed at 2.7 W/kg and 2450 MHz (Mitchell et al. 1988, 1989). MW exposure may also change innate locomotor behavior. D'Andrea et al. (1979) found changes in natural behavior measured by stabilimetric activity, decreased locomotor behavior, at 1.23 W/kg and 2450 MHz. Conversely, Mitchell, Switzer, and Bronaugh (1977) found an increase in locomotor behavior at 2.3 W/kg and 2450 MHz.

A number of behavioral studies addressed pulsed MW radiation. Frey, Feld, and Frey (1975) reported distinct differences in a single behavioral end point in rats exposed to pulsed (0.2 W/kg) or CW (2.4 W/kg) radiation at 1200 MHz. Lebovitz (1981) found a considerably higher effective SAR, around 3.6 W/kg, for rats exposed to a 1300-MHz field. Raslear and colleagues (1993) found changes in cognitive function in male rats exposed at high peak powers. In a small sample of rats exposed near their whole-body resonance frequency of 600 MHz, average SARs of 0.4 and 4.1 W/kg did not induce work stoppage (D'Andrea, Gandhi, and Lords 1977). Pulsed exposure of monkeys at 1300 MHz and SARs between 0.05 and 0.8 W/kg did not produce any significant differences in behavior (D'Andrea, Cobb, and de Lorge 1989), while 4.5 W/kg disrupted behavior (de Lorge 1984). Akyel (1991) and colleagues suggest that the threshold for effects at 1250 MHz is less than 23 W/kg and approaches 7.6 W/kg. Hence, most of the available information does not indicate significantly reduced effective SARs for pulsed exposures, although this analysis does not conside the potential influence of PRF and pulse width.

Possible reasons for some of the inconsistent findings in behavioral studies include experiments performed near the threshold for effects at a given frequency for a given species; use of a small number of animals; use of different species and strains of test animals; behavioral evaluation after MW exposure versus evaluation during exposure; method of

radiation delivery, e.g., locally to the top of the head versus frontally to the whole body; high-peak SARs possibly inducing MW hearing; type of operant task evaluated; differences due to sampling error or some artifact; and other differences in experimental methods. Obviously, many of these differences translate to experimental limitations that may make the experimental findings difficult to interpret.

The threshold for behavioral responses is not only associated with a significant increase in body temperature due to absorbed RF energy, it also appears to be frequency dependent. Experiments with rats and monkeys at multiple frequencies and SARs have demonstrated the importance of geometrical resonance in establishing the lowest effective SARs for measured end points. De Lorge (1984) demonstrated that SAR thresholds for behavior disruption in monkeys varied with frequency. The thresholds were 3.2 W/kg at 225 MHz, 4.5 W/kg at 1300 MHz, 4.7 W/kg at 2450 MHz, and 8.4 W/kg at 5800 MHz. D'Andrea, Gandhi, and Lords (1977) found that the near-resonance frequency of 600 MHz produced the shortest times to work stoppage in male Long-Evans rats, and this appeared to be SAR dependent. At SARs of 4 and 6 W/kg, rats completed the experiment without work stoppage. Time to work stoppage was inversely associated with SAR at 8 and 16 W/kg. Lebovitz (1981) suggested that SAR thresholds near 3.6 W/kg exist for effects observed with female rats exposed to pulsed microwaves at 1300 MHz. D'Andrea and colleagues (1986b) observed the threshold for effects between 0.14 and 0.7 W/kg for male rats chronically exposed to CW, 2450 MWs, although effects were more clearly established at the higher SAR.

Although analysis using whole-body SAR appears to be an adequate predictor of the threshold for behavioral effects, de Lorge (1983) suggests that other facets of energy absorption may be more important "such as distribution or local resonance might be more consistent parameters for predicting behavioral effects." Also, as indicated earlier, rectal temperature change of more than 1°C may be a more useful predictor in the laboratory. However, this assumes that the actual cause of the behavioral changes is whole-body temperature elevation, which may not be the case. Again, turning to the thoughts of de Lorge (1983), it is possible that "the causal agent could be energy deposition in the brain or head area."

3.3.5 Effects on Reproduction, Development, and Growth

The potential for RF-induced reproductive and developmental effects has been evaluated, primarily at frequencies of 2450 and 27.12 MHz, and in the VLF and LF parts of the spectrum. These spectral regions are important because of the large number of sources that operate at these frequencies and bands. 2450 MHz is used in microwave heating in both public and private sectors. Results of a number of studies at this frequency are in Table 3-8. Many 27-MHz sources are used in industry and medicine, including dielectric heaters, plasma processors, diathermy and hyperthermia devices; 27 MHz is also used in the public sector in communications devices. Table 3-9 is a compilation of studies at 27 MHz. VLF and LF frequencies have been studied because of concern about RF emissions from cathode-ray-tube type televisions and visual display terminals. Literature reviews of studies of reproductive effects are available (O'Connor 1980, 1990; Elder and Cahill 1984; NCRP 1986; Michaelson 1986; Michaelson and Lin 1987; Lary and Conover 1987; Chiang and Shao 1989).

3.3.5.1 Reproductive Effects

The testes were the first reproductive structure studied. Testicular damage is an obvious end point for study because of the thermal sensitivity of that gland. Generally, studies showed that RF exposure can produce degenerative changes when temperatures were elevated (Imig, Thomson, and Hines 1948; Prausnitz and Susskind 1962; Ely, Goldman, and Hearon 1964). However, the method of

Table 3-8. Studies of Reproductive and Developmental Abnormalities at 2450 MHz

Species	SAR (W/kg)	Power Density (mW/cm^2)	Effects	Reference(s)
Rats (male)	NR	80 (locally to scrotum)	Degenerative effects to testicular tissue	Muraca, Ferri, and Buchta 1976
Rats (male)	0.9 to 4.5[a]	5	No significant differences on reproduction	Berman, Carter, and House 1980
	2[a]	10	No significant differences	
	5.6[a]	28	Temporary sterility	
Rats (male)	9	NR	No significant MW effects	Lebovitz and Johnson 1987
Rats (male)	0.15 to 0.4	0.48	Increased testicular mass at 13 months but not at 25 months	Johnson et al. 1984
Rats (female)	28 to 34	NR	No observed malformations, more maternal deaths and increased resorptions	Chernovetz, Jastesen, and Oke 1977
Rats (female)	3.6 to 5.2	20	No significant differences in malformations	Jensh, Weinberg, and Brent 1983
Rats (female)	3.6 to 5.2	20	No differences in neonates in five behavior tests; exposed females were more active than exposed males	Jensh, Vogel, and Brent 1983
Rats (female)	2 to 3[a]	10	No differences in litter size; significant differences in body/brain weights	Shore, Felten, and Lamanna 1977
Rats (female)	4.2[a]	28	No significant differences	Berman, Carter, and House 1981

Table 3-8. (Continued)

Species	SAR (W/kg)	Power Density (mW/cm^2)	Effects	Reference(s)
Hamsters (female)	6[a] 9[a]	20 30	No significant differences; increased resorptions and malformations; decreased fetal weight	Berman, Carter, and House 1982a
Mice (female)	38 ± 3	NR	No significant effects due to MW radiation	Chernovetz et al. 1975
Mice	≤ 43 to ≤ 112[b]	≤ 123 (estimated)	Malformations, exencephalies; effects include resorption, stunting, and fetal death	Rugh et al. 1975
Mice (female)	80.8 to 217	NR	Largest number of malformations in microwave-exposed group; effects reduced in groups receiving anesthesia + MW	Rugh and McManaway 1976
Mice (female)	2 to 22.2	3.4, 13.6, 14, 28	Statistically significant increase in cranioschisis with sum of data collapsed across MW-treatment groups	Berman, Kinn, and Carter 1978
Mice (female and offspring)	16.5 ± 4.5	28	Significant differences in body weight and immature skeletal development	Berman, Carter, and House 1982b
Mice (female)	23.4 to 40.2	30	Significant increase in malformations, cleft palate observed most frequently	Nawrot, McRee, and Staples 1981

Table 3-8. (Continued)

Species	SAR (W/kg)	Power Density (mW/cm^2)	Effects	Reference(s)
Mice (female)	40.2	30	No differences in brain cholinesterase activity or malformations	Nawrot, McRee, and Galvin 1985
Mice (female)	0.48[c,d]	NR	Significant differences in number of pyknotic cells in embryos	Fukui et al. 1992
Mice pups	113 to 93 (dams) 117 to 122 (pups)	NR NR	Significant differences in body weight for both sexes	Rugh 1976b
Rat pups	9 to 10[e]	40	Increased adrenal weights, no differences in growth rates	Guillet and Michaelson 1977
Rat pups	0.9 to 4.7	5	Increased response of lymphocytes to mitogen stimulation	Smialowciz, Kinn, and Elder 1979
Squirrel monkeys and offspring	3.4[f]	NR	Increased infant mortality	Kaplan et al. 1982
Squirrel monkeys and offspring	3.4[f]	NR	No differences in infant mortality	Kaplan 1981
Mice pups	16.5[f]	28	Reduced brain weights	Berman, Carter, and House 1984
Rat pups (post- and prenatal exposure)	2 to 4[f] 16.5 to 5.5[g]	10	Differences in swimming endurance times, startle responsiveness, and body weight (see text)	Galvin et al. 1986

Table 3-8. (Continued)

Species	SAR (W/kg)	Power Density (mW/cm^2)	Effects	Reference(s)
Rats (female)	NR	1.5 to 2	No differences in abnormalities; lengthened estrus cycle	Earle and Blake 1985
Chicken	NR	200 280 400	Abnormalities and death when temperature approached 55°C	Van Ummersen 1961
Quail	14	30	No differences in most bloodborne end points; no differences in malformations	Hamrick and McRee 1975
Quail	14	30	No significant differences	McRee et al. 1975
Chicken	NR	1.4 to 6.2	Differences in rate of development in 4-day and 5-day exposed eggs	Fisher, Lauber, and Voss 1979
Quail	3.3 to 3.8 13.2 to 15.2	5 20	Increased growth rates	Spiers and Baummer 1991
Chicken	2.9	3.6	Reduced hatchability in group receiving greatest exposure duration	Braithwaite et al. 1991

[a]Estimate from Elder and Cahill (1984).
[b]The SA was estimated by the authors, and exposure durations were reported as < 5 minutes, so estimates of SAR are given as ≤ 5-minute values.
[c]Maternal SARs.
[d]SAR does not represent whole-body exposure since the head and neck region was shielded with reflective material during exposure.
[e]SARs were cited as 9 to 10 W/kg, but the EPA (Elder and Cahill 1984) estimates substantially higher values, between 20 and 60 W/kg.
[f]Estimated fetal SARs.
[g]Postnatal SARs.
NR, not reported.

Table 3-9. Studies of Reproductive and Developmental Abnormalities at 27.12 MHz with Rats

SAR (W/kg)	Field Strength (V/m)	Field Strength (A/m)	Effects	Reference(s)
0.007 to 0.05[a]	220 to 670	0.1 to 0.3	Reduced number of matings and reduced conception after mating	Brown-Woodman et al. 1989
11.1 to 12.5	300	55	Increased postimplantation deaths and resorption; increased visceral, skeletal, and external malformations	Lary et al. 1982
~11	300	55	Embryotoxic and teratogenic at elevated temperature (41 and 42°C versus 38.1°C), especially with body temperature maintained for longer exposures; malformations restricted to head	Lary et al. 1983
~11	300	55	Threshold temperature for birth defects and prenatal death = dam's colonic temperature 41.5°C	Lary et al. 1986
0.00011[b]	20	0.05	Increased resorptions, reduced body weight increase, incomplete cranial ossification	Tofani et al. 1986
NR	NR	NR	Resorptions and malformations increased with increasing rectal temperatures	Brown-Woodman et al. 1986
2.8[c] 4.2 5.6	5 W/cm^2[d] 10 W/cm^2 15 W/cm^2		Embryolethality at PRF = 10 Hz for 60 minutes (2.8 W/kg); no differences in fetal weight, external malformations, or core temperature	Brown-Woodman and Hadley 1988
NR	NR	NR	Effects dependent upon pregnancy phase; malformation frequency directly related to rectal temperature	Dietzel 1975

[a]Because of the spatial inhomogeneity of the field near the electrodes and gang exposure methods, only very crude dosimetric estimates can be made, and these must be used with caution. Estimated SARs are based on the extremes of the measured field strength. The basis for these estimates is information in Durney, Massoudi, and Iskander (1986).
[b]This is an upper limit of possible SARs.
[c]SARs were determined using a saline-filled model of a 300-g rat housed in the exposure cage.
[d]Pulsed at PRF = 10, 20, or 30 Hz.
NR, not reported; PRF, pulse repetition frequency.

temperature measurement in some of these studies could confound the results because of the use of metallic needles and thermocouples that could modify the local field.

Mikolajczyk (1976) found no changes in testicular weights in rats (2860 to 2880 MHz, 10 mW/cm^2, SARs ~ 1 to 2 W/kg) (Elder and Cahill 1984). Muraca, Ferri, and Buchta (1976) compared testicular effects produced by local heating at 2450 MHz or scrotal immersion in heated water. Both treatments produced similar histologic damage to testicular tissue. The researchers suggested that some of the MW effects may not be associated with thermal processes, but this was inconclusive. Michaelson and Lin (1987) suggested that differences observed by Muraca could be due to "different heating rates or thermal gradients produced by the two heating modalities." In a long-term study of Sprague-Dawley rats, testicular mass was increased (marginal statistical significance, $P = 0.04$) in exposed animals at 13 months but not at 25 months (Johnson et al. 1984). Hence, MW radiation cannot be viewed as reliably affecting testicular mass in this study.

Male Sprague-Dawley rats exposed at 5.6 W/kg (see Table 3-8) demonstrated temporary sterility. No differences were found in body and organ (testes, liver, adrenals) weights and sperm concentrations (Berman, Carter, and House 1980). Smialowicz et al. (1981) studied sperm mutagenesis, observing no differences in a dominant lethal assay in male Sprague-Dawley rat pups exposed (100 MHz, 2.5 to 3 W/kg).

The potential for microwave-induced effects on reproductive hormone concentrations has been examined. A single 8-hour session at 1.3 GHz (average SAR = 9 W/kg) in a cylindrical waveguide section produced a 4.5°C rise in rectal temperature in unrestrained male rats. There were no differences in daily sperm production and follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels. Both seminal vesicle weight and epididymal sperm count were significantly different on day 26 but not at the other three sampling periods. The authors conclude "that the clearly thermogenic dose was not sufficient to induce a critical temperature rise in the testes of unrestrained rats" (Lebovitz and Johnson 1987).

Rugh and colleagues found that female mice were more sensitive to 2450-MHz MW irradiation than males and that the lethal dose in females showed an estrus cycle dependence (Rugh et al. 1975; Rugh 1976a). Earle and Blake (1985) found that the estrus cycle lengthened in exposed females but observed no differences in reproductive capability. At a substantially lower frequency (2 kHz), Baumann and coworkers (1989) restrained and exposed female rats to a 2-mT magnetic field after the animals received an implantation of mammary adenocarcinoma near the lower nipples. Concentration differences of prolactin and LH were statistically significant. Levels of FSH approached significance, while estrogen and progesterone were not different. In a replicate experiment, there were no significant differences in concentrations of LH, FSH, and prolactin. Data from vaginal smears, obtained from both experimental groups, demonstrated no differences.

Potential effects on reproductive capacity and performance have been evaluated in multigenerational studies. No reproductive effects were found in two female dogs exposed to 24-GHz microwaves (24 mW/cm^2), and in two generations of mongrel dogs exposed to pulsed MW (width = 3 μs, PRF = 360 Hz) at 1285 MHz. In the latter experiment, SARs were 1 W/kg (20 mW/cm^2), 2.5 W/kg (50 mW/cm^2), or 5 W/kg (100 mW/cm^2) (Michaelson, Howland, and Deichmann 1971).

In another multigenerational study, Jensh (1984a, 1984b) exposed pregnant Wistar rats to 6-GHz microwaves at 35 mW/cm^2 (SAR ~ 7.3 W/kg). Maternal weight gain of the irradiated rats was significantly less than concurrent controls at day 21 of gestation. The average number of fetuses per F_{1a} (in utero exposure group) litter was lower for the irradiated dams. When rebred to produce the F_{1b} (second) generation, irradiated dams weighed less than concurrent controls but had a greater weight change throughout pregnancy. There were no differences in the average F_{1b} litter size.

Jensh, Weinberg, and Brent (1983) performed a similar experiment at 2450 MHz (see Table 3-8). Dams were exposed 6 h/d throughout pregnancy. At age 90 days, half of the F_{1a} generation was bred to measure reproductive ability. Male and female animals were bred within that group and with controls. The average litter size and the initial average maternal weights were significantly different when both parents were control animals. Final average maternal weights were not different.

Brown-Woodman et al. (1989) evaluated the reproductive function and fertility of female Sprague-Dawley rats exposed at 27 MHz (see Table 3-9) and performed two duplicate trials. Females were mated with unexposed males 3 days after exposure was ceased. RF-exposed animals exhibited a reduction in mating and a reduction in pregnancy in both experiments. When the data from both trials were combined, the finding was statistically significant.

3.3.5.2 *Developmental Effects*

A number of studies have demonstrated that RF fields are embryotoxic and teratogenic. The following review will consider mammalian effects at 2450 and 27.12 MHz, VLF and LF regions, and other selected frequencies. Mammalian species that have been studied include mice, rats, hamsters, and monkeys. Effects on avian embryos will be addressed separately.

In reviewing available teratology data, the reader should keep in mind the classification scheme for developmental end points proposed by Frankos (1985) and modified by Thomas and Ballantyne (1990). These end points are shown in Table 3-10. This scheme classifies developmental effects into either type I or type II changes. Type I changes are irreversible, life-threatening changes that are usually associated with gross malformations. Type II changes are reversible, non-life-threatening, and are not related to malformations.

3.3.5.2.1 2450 MHz The frequency of 2450 MHz is supraresonant for the rat, which means that energy absorption is less than optimum. In the studies reviewed in Table 3-8, there were no reports of increased malformations in the rat at SARs from 4 to 40 W/kg at 2450 MHz. Increased resorptions, a type I change, were observed at a SAR of 31 W/kg for 20 minutes, which produced an average rectal temperature of 42 ± 1°C (Chernovetz, Justesen, and Oke 1977). One group of researchers noted reduced postnatal body weights for pups exposed in utero at maternal SARs of 2 to 3 W/kg, although increased ambient temperature during pregnancy confounds interpretation (Shore, Felton, and Lamanna 1977). EPA researchers saw no effects on fetal weights at a maternal SAR of 4.2 W/kg, and postirradiation colonic temperature of 40.3 ± 0.4°C (Berman, Carter, and House 1981). Jensh, Vogel, and Brent (1983) found increased activity in first-generation females where the dams' SARs ranged between 3.6 and 5.2 W/kg.

Table 3-10. Developmental End Points

Type I Changes	Type II Changes
Reduced live births	Reduced birth weights
Reduced live fetuses	Reduced postnatal survival
Increased resorptions	Reduced postnatal growth, reproductive capacity
Increased fetal malformations	Increased fetuses with retarded development

Adapted from Frankos (1985); and Thomas and Ballantyne (1990).

The frequency of 2450 MHz is near resonance for hamsters and mice, so energy absorption should be enhanced. Berman, Carter, and House (1982a) detected a number of type I changes including increased resorptions and fetal death at a SAR = 9 W/kg, which elevated mean rectal temperature to around 40°C. No differences were seen at 6 W/kg. Increased resorptions and fetal death were observed in mice when dams were exposed between 81 and 217 W/kg, producing a 40.8°C rectal temperature. Effects were greatly attenuated at the same SAR range when the animals were treated with a hypothermic anesthetic (Rugh and McManaway 1976). An average SAR of 16.5 W/kg for 100 min/d produced a marginally significant delay in development, a type II change (Berman, Carter, and House 1982b). Berman, Kinn, and Carter (1978) found significant abnormalities in the low-SAR group, but not in three groups treated at higher SARs. When these data were collapsed across all treatment groups, a significant increase in cranioschisis was noted. However, a biologic gradient was not observed with SAR, and collapsing the data across dose rates does not provide convincing evidence that MW radiation reliably affected this end point. Nawrot, McRee, and Staples (1981) noted type I effects—reduced implantations and increased abnormalities—at SARs between 23.4 and 40.2 W/kg, which increased colonic temperatures an average of 2.3°C. In a second report, these effects were not observed at a local uterine SAR of 40 W/kg, for a 2.3°C colonic temperature rise (Nawrot, McRee, and Galvin 1985). Fukui et al. (1992) observed an increase in pyknotic cells in embryonic brain tissue of mice treated with microwaves (core temp. = 42.5°C) or hot water (42°C) on day 13 of gestation, and a statistically significant increase in embryo death in the MW-treated animals in comparison with controls. The authors attribute the effects to thermal stress.

3.3.5.2.2 27.12 MHz The frequency of 27.12 MHz is highly subresonant for rats, the test animal used in all the reviewed studies. Statistically significant reductions in mating and pregnancy were seen in female rats (Brown-Woodman et al. 1989). Gang-exposure techniques used in this experiment and the proximity of irradiated animals to the source do not allow the SAR to be reliably estimated. Two studies reported that developmental effects depended upon the phase of pregnancy in which the dams were irradiated (Dietzel 1975; Lary et al. 1982) and that adverse developmental effects were directly related to the maternal rectal temperatures (Dietzel 1975; Lary et al. 1982, 1983, 1986; Brown-Woodman et al. 1986). Dietzel (1975) observed a biologic gradient for malformations as a function of temperature, while Lary and colleagues (1986) established embryotoxic and teratogenic thresholds in defining a dose–response curve. The incidence of embryo death and malformations exhibited a dramatic increase when the dam colonic temperature exceeded 41.5°C, as shown in Fig. 3-5. Again, malformations were primarily to the head. Lary et al. (1983) demonstrated that the intensity of the observed teratogenic effects was associated not only with the dam's colonic temperature but also the time that the temperature remains in an elevated state.

Tofani et al. demonstrated type I and II changes at an extremely low SAR estimated to be less than 0.00011 W/kg (Tofani et al. 1986). Lary (1991) analyzed the study by Tofani and provided information on four important points: (1) The 27.12-MHz exposure system used produced near-field exposures of the test animals, which possibly could produce capacitive coupling between the test animals and the antenna. At NIOSH, Lary exposed rats in a TEM cell, estimating whole-body average SAR, based on field-strength values in the cell, to be around 0.2 W/kg. However, the animal bodies, being very close to the source, capacitively coupled to the source, which produced very high current densities in the tails of the rats. This led to local SARs around 1000 W/kg, and local temperatures were elevated to 50 to 60°C, which "cooked" the tails of the experimental animals. In some cases, the animals lost their tails about 1 to 2 days after exposure. Obviously, under these conditions the animals were severely stressed,

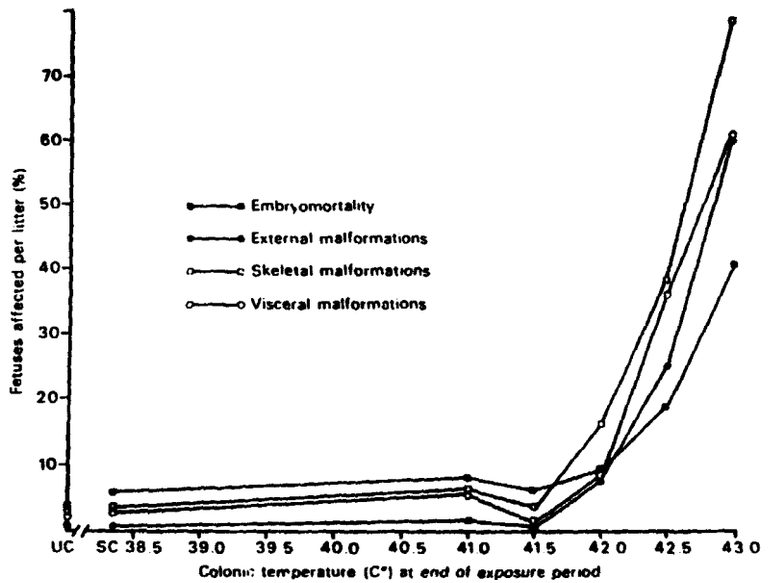

Figure 3-5. Rat embryo death and malformations as a function of colonic temperature of dams exposed at 27.12 MHz for 10 to 40 minutes. From Lary et al. (1986); used with permission.

although this would not be indicated by estimates of the average SAR. (2) In regard to temperature measurement, it is not clear if the rectal temperatures were measured continuously during exposure in a small number of animals, or if the animals were removed from the field to sample temperatures. Both treatments can induce a stress response in the animals. Also, calibration of the thermometry system is not detailed. (3) It does not appear that blind methods were used in the interpretation of teratologic end points. (4) There is an inconsistency in the birth-weight data and data on incomplete cranial ossification. Here, there is a trend of decreasing birth weight that is consistent with the observations of increased incomplete cranial ossification. The inconsistency resides with the relative magnitudes of these effects. The differences in birth weights are not statistically significant. However, the differences in incomplete cranial ossification are highly significant for all exposure groups.

Lary (1991) hypothesized that the observed effects were stress-induced and offered the following scenario as a possible explanation. The experimental animals were capacitively coupled to the source, which produced high local levels of current density and SAR. This stressed the animals, altering the normal hormonal physiology of the dams. Eggs were able to implant successfully in the uterus but were unable to develop during post-implantation because the uterus could not support embryonic development. The effects on cranial ossification and body weight represent a stress-induced delay in development.

Although the findings by Tofani et al. (1986) cannot and should not be dismissed, in light of the number of questions dealing with

dosimetry and study methodology (Lu and Michaelson 1987; Lary 1991; Tofani et al. 1987), interpretation must be approached with circumspection. The reported effects are not unique to this study and are biologically plausible with RF radiation. However, effects at the reported SAR cannot be viewed as reliably coherent with known interaction mechanisms and dosimetric facts or reasonably anticipated outcomes until there has been an independent experimental replication.

Brown-Woodman and Hadley (1988) exposed pregnant rats to pulsed fields generated by shortwave diathermy units. SARs varied with the PRF as noted in Table 3-9. Interestingly, only one type of diathermy unit produced measurable SARs in the model, although both units had similar operational parameters. Animals received single exposures on day 9 of gestation for 30, 45, or 60 minutes depending upon the PRF. Rectal temperatures (measured before and after exposure), average fetal weight, and external malformations were not significantly different between the groups. Increased resorption percentage was noted with one diathermy unit but not with the other. For the biologically effective unit, resorption percentage varied directly with duration of exposure and inversely with SAR and average power density. Resorption percentage was similar to controls when a 5.6-W/kg SAR was maintained for 30 minutes at a PRF = 30 Hz. Slight increases occurred when dams were exposed 45 minutes at a SAR = 4.2 W/kg (PRF = 20 Hz), and marked increases were noted for 60-minutes exposures at 2.8 W/kg (10 Hz). Conversely, for the biologically ineffective unit, exposures for 60 minutes (15 Hz) or 45 minutes (26 Hz) produced a lower resorption percentage than experienced by control animals (Brown-Woodman and Hadley 1988). The authors do not offer an explanation of this result. However, they do discuss the potential for localized temperature increases within the rat body. This would be more likely to produce the observed differences between the two exposed groups if the applicators of the diathermy units were substantially different but only one type of applicator was used (130-mm-diameter rigid electrodes). It is possible that the exposure within the near field of this source produced capacitive coupling between the animals and the applicators, as discussed earlier. However, localized temperature increases would probably not explain the lack of measurable SAR in the saline-filled model for one diathermy unit. Interpretation of these results has been made difficult because of their equivocal nature.

3.3.5.2.3 VLF-LF VLF-LF studies have used pulsed magnetic fields with rectangular and sawtooth pulse shapes. A sawtooth-pulse is triangular but skewed in one direction, as shown in Fig. 3-6. In general, a strength of these studies is the use of multiple doses, and the use of flux density values that approximate the exposure levels they are attempting to model, operator exposure near VDTs. In mammalian studies, the frequency studied, 20 kHz, is near the primary operational frequency of the high-voltage transformer of CRT-type VDTs.

Two studies reported statistically significant differences in type I end points (Tribukait, Cekan, and Paullson 1986a, 1986b; Juutilainen and Saali 1986). Only the study by Tribukait and colleagues found a statistically significant effect in mammals, but this study has been criticized for use of the fetus, not the litter, in statistical analysis. These results were not replicated by others using similar magnetic fields (Stuchly et al. 1988; Frolen and Svedenstal 1989; Wiley et al. 1990).

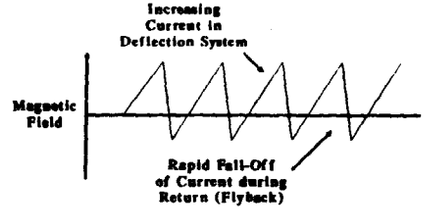

Figure 3-6. Sawtooth pulse associated with the horizontal deflection system of a cathode-ray tube video display terminal.

Stuchly et al. (1988) exposed female rats to 18-kHz magnetic fields in the form of sawtooth pulses. These had peak-to-peak levels of 0, 5.7, 23, or 66 μT. The authors state that the 5.7-μT level is about twice the flux density experienced by VDT operators at a distance of 30 cm, when dimensional scaling factors are used to adjust for differences in the maximum-induced currents between rats and average man. Animals were exposed 7 h/d, 15 days prior to pregnancy to day 22 of gestation. Abnormalities were classified as major malformations, minor anomalies, or common variants. Minor skeletal anomalies were significantly increased for the highest exposure group when analyzed by fetus but not when analyzed by litter. Two types of common skeletal variants were significantly increased at the two highest flux densities when analyzed by fetus or litter, which was characterized as typical teratologic "noise." This is supported by the average fetal weight data, which were not significantly different. Furthermore, fetal weights were higher and less variable for the exposed groups in comparison with the controls.

Female rats were exposed to a 20-kHz, 15-μT (p-p) sawtooth magnetic field, 24 h/d for 20 days. There were no significant differences in implantations, pre- and postimplantation losses, resorptions, malformed fetuses, minor malformations, living fetuses, and measures of dam and fetal body masses. The RF-treated group had more skeletal variants and minor skeletal anomalies than controls, which "are common in teratological studies." This finding was statistically significant when analyzed by the fetus but not when analyzed by the litter. The authors' interpretation is that it is possible that low-frequency magnetic fields may have an effect on ossification (Huuskonen, Juutilainen, and Komulainen 1993).

Frolen found a significant increase in fetal malformations in mice exposed to pulsed magnetic fields but was unable to replicate this result in a later study with CBA mice (20-kHz, $B = 15$ μT). However, there was an increase in resorptions (Frolen and Svedenstal 1989; Juutilainen 1991). No effects on reproductive ability, metabolism, and growth were seen at 25 kHz (Bollinger, Lawson, and Dolle 1974). Although just outside of the lower VLF boundary (3 kHz), no effects were seen on reproductive hormones in rats at 2 kHz with a 2-mT magnetic field (Baumann et al. 1989).

In a study at the University of Toronto, CD-1 Swiss Webster-derived mice were exposed to 20-kHz sawtooth pulses at peak-to-peak (p-p) magnetic flux density levels: 3.6, 17, and 200 μT. These equate to rms values of 1.1, 5.1, and 60 μT. Exposures were designed to bracket potential VDT operator exposures at 30 cm. The 3.6-μT level was selected "to correspond, after the application of current-induction-based scaling considerations, to actual exposure levels likely to be experienced by VDT operators" (Wiley et al. 1990); 17 μT is similar to the level used by Tribukait and Frolen, cited earlier. One hundred ninety-two animals were used in four replicate experiments within each exposure group and the sham group. Exposures were from day 1 to day 18 of gestation, 20 to 21 h/d. Among all groups, there were no statistically significant differences in these major end points: embryo/fetal mortality, fetal malformations (externa, visceral, and skeletal), and fetal growth (Wiley et al. 1990). At this writing, the results of this study have not received peer review prior to journal publication. However, the study design included an audit committee that provided a scientific review.

3.3.5.2.4 Other Frequencies Researchers at NIOSH reported a combined teratogenic effect with exposure of pregnant rats on day 13 of gestation to 10-MHz RF and the solvent 2-methoxyethanol (2-ME). An initial RF SAR of 6.6 W/kg was used to elevate rectal temperature to 42°C, then temperature was maintained for 30 minutes during which time the SAR was 0.8 to 6.6 W/kg. 2-ME was administered by gastric lavage 5 minutes prior to RF exposure. Treatment with RF + 2-ME did not significantly affect viability and fetal weight, but it did affect fetal malformation percentage (Nelson et al. 1991).

Pregnant Sprague-Dawley rats were used in an experiment at 100 MHz (average SAR = 0.41 W/kg, 25 mW/cm^2). No differences

between exposed and control animals were observed in maternal weight and temperature. However, both groups exhibited around a 10-g weight loss. This was attributed to lack of available food and water for animals with a relatively high metabolic rate. Embryotoxic evaluation showed a statistically significant decrease in the percentage of live fetuses with minor skeletal variations per litter in the RF-exposed group. There were no significant differences in external malformations and major skeletal abnormalities (Lary, Conover, and Johnson 1983).

EPA researchers exposed pregnant rats to circularly polarized microwaves (970 MHz) with whole-body SARs of 0.07, 2.4, or 4.8 W/kg for 22 h, from day 1 through day 19 of gestation. No end point (pregnancy rates; preimplantation losses; live, dead, resorbed, or total fetuses; fetal weights, fetal skeletal maturity or postimplantation losses) was reliably affected at the lower two SARs. At 4.8 W/kg, fetal body weight was significantly lower than observed in sham-exposed fetuses. The average number of ossified sternebrae was lower at 4.8 W/kg than for sham-irradiated animals and for exposed litters at the other two SARs. Measurements of dam rectal temperatures were not made, so no reliable conclusions dealing with the thermal nature of the exposures could be made. The number of exposed animals included in the high-dose-rate group was small. However, the authors claim that "application of a multiple comparison t test on gain of body weight in all nonpregnant, sham-irradiated rats vs. nonpregnant rats in each exposure level demonstrated that only the rats receiving 4.8 W/kg had significantly lower gain in body weight ($p < 0.05$)" (Berman et al. 1992).

Jensh (1984a) exposed gravid Wistar rats at 7.3 W/kg and 6 GHz daily throughout pregnancy. There were no differences in developmental abnormalities between exposed fetuses and the controls. Fetal weight of the irradiated group was significantly less than the control groups, while the sham-exposed group weighed significantly more than the home-cage and anechoic-chamber controls.

3.3.5.3 Growth and Other Postnatal End Points

Rugh (1976b) found no evidence that in utero exposure (Table 3-8) might modify the radiosensitivity of mice pups. Rat pups exposed at >9 W/kg at 2450 MHz demonstrated a nonspecific stress reaction, similar to pups injected with ACTH (Guillet and Michaelson 1977). An increase in the mitogen-stimulated lymphocyte response occurred in rat pups receiving perinatal exposure (Smialowicz, Kinn, and Elder 1979). Infant mortality in squirrel monkeys was elevated in one experiment but not in a replicate (Kaplan et al. 1982; Kaplan 1981). Galvin and colleagues (1986) noted a significant reduction in swimming endurance, although it appeared to be reversible. Prenatal SARs were 2 to 4 W/kg, while in the postnatally exposed groups, they were 5.5 to 16.5 W/kg. EPA scientists observed that exposed rats (100 MHz, 2.5 to 3 W/kg) exhibited earlier eye opening and had higher body weights than sham-exposed offspring (Smialowicz et al. 1981). EPA researchers also found decreased brain weights and body weights in mice pups exposed prenatally at an average maternal SAR of 16.5 W/kg (Berman, Carter, and House 1984). Jensh, Vogel, and Brent (1983) found that MW-exposed (2450 MHz, 3.6 to 5.2 W/kg) neonates were significantly heavier than their sham-exposed counterparts through week 8 of life.

Jensh (1984a and b) exposed 10 pregnant Wistar rats at 35 mW/cm² and 6 GHz (SAR ~ 7.3 W/kg) for days 12 to 14 of gestation in a multigenerational study. Growth and growth rate of exposed animals were significantly different until postnatal week 5, after which there were no differences. Significant differences were found in a number of reflex and behavioral tests. In a critique, O'Connor (1990) pointed out that a small number of litters were used in the statistical analysis.

Neonatal mouse pups were exposed at 148 MHz (SAR ~ 0.013 W/kg, 63.3 V/m) for 10 days. Six hundred days later, there were no differences in body weight, growth rate, hematocrit, hemoglobin, erythrocyte, leukocyte, and differential cell counts (Lin, Nelson, and Ekstrom 1979). Bollinger, Lawson, and Dolle (1974) exposed mice at 25 kHz to field strengths of either 15 kV/m and 7.5 A/m, or 10.6 kV/m and 5.3 A/m, for 1 h/d, 5 d/wk, for 50 hours. No significant effects were found on reproductive ability, metabolism, or growth.

3.3.5.4 Nonmammalian Species

Van Ummersen (1961) exposed chicken eggs at 48 hours of development. Exposure parameters are in Table 3-8. Egg temperature and thermal gradients were monitored by insertion of a hypodermic needle thermistor probe. Although the materials of construction are not specified, it is possible they were conductive, which would modify the field distribution within the egg. Following exposure, eggs were maintained in the incubator at 39°C until 96 hours of development. Irradiation appeared to affect 121 embryos. Morphologic abnormalities and death of embryos were found when the MW-induced temperature reached around 55°C.

Hamrick and McRee (1975) observed no gross malformations in hatchlings and no differences in body weight, organ weights (heart, liver, gizzard, adrenals, and pancreas), WBC and RBC counts, lymphocytes, hematocrit, monocytes, eosinophils, basophils, and heterophils. A marginally significant reduction (3.3%) in hemoglobin was observed in MW-exposed animals. In another study, the outcome was similar for weights, malformations, and bloodborne end points (McRee et al. 1975).

Fisher, Lauber, and Voss (1979) heated chicken eggs to between 32 and 36°C with 2450-MHz microwaves, finding that the development rate of exposed animals was augmented in comparison with controls. Spiers and Baummer (1991) also found that 2450-MHz microwave exposure increased growth rates in Japanese quail embryos. Braithwaite et al. (1991) observed a nonsignificant reduction in the hatchability of chicken eggs exposed from days 0 to 19 of incubation. No differences were seen for eggs exposed from day 0 to either day 7 or 14 of incubation. Kondra and colleagues (1970) report no differences between control and exposed (6 GHz) groups in average egg weight, fertility, and mortality. In the high (400 pW/cm²) and low (0.02 pW/cm²) continuous-exposure groups, egg weight was significantly less than controls. These animals apparently ovulated more frequently, producing more low-weight eggs, but the total egg mass produced was not significantly different from controls.

Juutilainen and Saali (1986) exposed chicken eggs to a sinusoidal magnetic field during the first 48 hours of development. RF and near-RF frequencies of 1, 10, and 100 kHz were used at flux densities of 0.13, 1.3, 13, and 130 μT. Evaluation for stage of development showed that 13 percent of the control eggs were abnormal. Significant differences in development between exposed and control eggs were found at all three frequencies at flux density values ≥ 1.3 μT, except for the trial at 10 kHz and 130 μT.

As discussed, studies of chicken embryo development showed effects at 2450 MHz and 1, 10, and 100 kHz. Effects at the higher frequency included an enhanced developmental rate, while at the lower frequency delayed development was demonstrated. These results are difficult to interpret in terms of human safety, primarily because the experiments examined development of an embryo in an egg membrane, not in the maternal body. Exposure of chicken eggs "obviously results in more independent and direct exposure of the developing organism than similar studies on mammalian organisms inside the maternal, also exposed, organism" (O'Connor 1990).

A number of studies have examined MW-induced effects in insects. Carpenter and Livstone (1971) report a decrease in the percentage of normal adult mealworm beetles exposed in a waveguide at 10.155 GHz for 30 minutes. When the exposure duration was 20 minutes, there were more normal adults in the MW-treatment group than in the waveguide controls. Olsen (1977) observed teratogenic effects when mealworm pupae were irradiated at 5.95 GHz, but at 4 GHz the results were similar to controls. Potential reproductive and

genetic effects were evaluated in male fruit flies exposed at 2450 MHz with power densities estimated to be 4.6, 5.9, and 6.5 W/cm^2. No significant effects were seen in reproductive or genetic outcomes (Pay, Beyer, and Reichelderfer 1972).

3.3.5.5 *Conclusions*

In summary, it has been established in animal experiments that exposures to RF fields, primarily at 27.12 and 2450 MHz, can produce adverse effects on reproduction, development, and growth in different mammalian species and in avian embryos. Most consistently, these effects appear to be related to hyperthermic conditions that depend on exposure intensity and duration. Typically, whole-body SARs must be in excess of 9 to 10 W/kg. The results of some studies are difficult to interpret because of use of the fetus and not the litter in the statistical analysis. According to Haseman and Hogan (1975), the litter is the preferred experimental unit in teratology studies.

Teratogenic and embryotoxic effects appear to be hyperthermic in nature and are associated with the timing of the exposure, the magnitude of the dam's rectal temperature, and the length of time that the rectal temperature is elevated. Even relatively brief exposures, on the order of minutes, can produce significant teratism if delivered at high levels on certain gestational days. Type I teratogenic effects most commonly observed include skeletal abnormalities of the head. Type II changes seen most frequently include reduced fetal weight, which "is used by the teratologist-toxicologist as an indicator of general health of the newborn" (O'Connor 1985). Typically, whole-body SARs that produce reduced fetal weight are of hyperthermic proportions. The report by Berman et al. (1992) is at a relatively low SAR (4.8 W/kg), but the number of animals used was small. Reversible effects on sterility and offspring behavior have been reported at lower SARs, but in no case do these levels approach SARs in currently recommended safety standards.

3.3.6 Endocrine and Neuroendocrine Effects

The neuroendocrine system is composed of the CNS and various glands including the hypothalamus, hypophysis (pituitary), thyroid, and adrenals. The endocrine system is the body's chemical regulatory system that is involved in the maintenance of homeostasis and the regulation of growth and metabolism. There are two major axes of the hypothalamus-hypophysis system. The hypothalamo-hypophyseal-adrenocortical (HHA) axis is involved in glucose, fat, and protein metabolism; electrolyte control; and the alarm reaction. The hypothalamo-hypophyseal-thyroid (HHT) axis is concerned with control of metabolism and oxygen consumption. Functional control is effected by hormones that are released into the circulatory system by the glands. The concentration of hormones varies with the body's circadian cycle to maintain homeostasis. Representative neuroendocrine effects, associated with microwave irradiation, are shown in Table 3-11.

Wright et al. (1984) found significant differences in the uptake of radioactive iodide-125 (^{125}I), ratio of thyroid ^{125}I to plasma ^{125}I, and thyroxine and triiodothyronine levels in rats. No significant differences were seen in protein-bound ^{125}I and plasma thyrotropin (TSH). Other researchers have not observed elevated thyroxine levels, although the frequency used in these studies was much higher (Kunz et al. 1984; Guy et al. 1980; and McRee et al. 1980). Parker (1973) observed no significant differences in thyroxine levels for three groups of rats exposed for 16 h, but found a significant depression for the group exposed at 15 mW/cm^2 for 60 hours.

Milroy and Michaelson (1972b) observed no differences in iodine uptake or thyrotropin. However, the study was unable to detect decreases in thyrotropin. Lu et al. (1981) found that thyrotropin levels in male rats varied inversely with power density and colonic temperature. Thyroid secretion rate was statistically elevated for local exposure of the thyroid but not for local exposure of the head of mongrel dogs (Michaelson et al. 1977).

Table 3-11. Neuroendocrine Effects

Species	Frequencies (MHz)	SAR (W/kg)	Average Power Density (mW/cm^2)	Duration (d×min)	Effects	Reference(s)
Rat (male)	28	0.5[a]	220	13×1416	Reduced ^{125}I uptake; reduced concentrations of thyroxine, TSH and triiodothyronine;	Wright et al. 1984
		0.4[a]	125	28×1380	no differences in adrenal weight	
Rat (male)	2450 Pulsed	0.15 to 0.4	0.48	750×1260	No differences in thyroxine or plasma cortosterone; significantly increased adrenal mass	Kunz et al. 1984; Guy et al. 1985
Rabbits	2450 CW	1.5 WBA 17 head	7 10	180×1380	No differences in cortisol or thyroxine	McRee et al. 1980
Rats	2450	3.8[b]	10, 20, 25	1×960	No differences;	Parker 1973
			15	2.5×1440	significant decrease in thyroxine and protein-bound iodine	
Rats (male)	2450	0.25 to 25[b]	1, 10, 100	1×10 up to 1×45	No differences between	Milroy and Michaelson 1972b
		0.25 to 2.5[b]	1, 10	56×480	exposed and controls in thyroid or thyrotropin activity	
Rats (male)	2450 120 Hz AM	8.4 to 14.7	40 to 70	1×60 or	Increased levels of corticosterone;	Lu et al. 1981
		8.4	40	1×240	decreased	
		0.21 or 2.1	0.1 or 1	1×240	levels of corticosterone	

Table 3-11. (Continued)

Species	Frequencies (MHz)	SAR (W/kg)	Average Power Density (mW/cm²)	Duration (d×min)	Effects	Reference(s)
Dogs	2450 CW 120 Hz AM	NR	20 to 40	1×60 (head)	No effect on growth hormone or serum thyroxine;	Michaelson et al. 1977
		58		1×60 (thyroid)	increase in serum thyroxine;	
Rats	2450 CW	6.3 12.6	30 60	1×60 1×60	decreased growth hormone levels	
Rats (male)	2450 CW	8 to 9.6 3.2 to 6.4	50 or 60 20 to 40	1×30 or 1×60 1×120	Increased colonic temperature and plasma corticosterone significantly elevated	Lotz and Michaelson 1978
Rat (male)	435 Pulsed	0.3 to 0.35	1	168×1320	Significant decrease in dopamine	Toler et al. 1988
Rat (male)	2450	0.15 to 0.4	0.48	180×1260 360×1260	No differences in thyroxine or plasma corticosterone;	Chou et al. 1985
	2450	2.5, 5 7.5	5, 10 15	42×1260	corticosterone levels decreased with higher environmental temperatures	
Rat (pups)	2450	9 to 10[c]	40	7×5	Increased adrenal weights; no differences in levels of corticosterone or adrenal responsiveness	Guillet and Michaelson 1977
Rats (male)	2450	1 to 1.5	5	80×480	No differences in adrenal mass	D'Andrea et al. 1979

Table 3-11. (Continued)

Species	Frequencies (MHz)	SAR (W/kg)	Average Power Density (mW/cm²)	Duration (d×min)	Effects	Reference(s)
Rats (male and female)	2860 to 2880 CW	1 to 2[b]	10	36×360	Changes in luteinizing hormone	Mikolajczyk 1976
Monkeys (male)	1290 Pulsed	2.1 3.0 4.1	20 28 38	1×480 1×480 1×480	Increased rectal temperature; increased cortisol at highest SAR	Lotz and Podgorski 1982

[a]SAR estimated, Durney, Massoudi, and Iskander (1986).
[b]SAR estimate from Elder and Cahill (1984).
[c]SARs of 9 to 10 W/kg were estimated by the researchers, but the EPA estimates SARs between 20 and 60 W/kg (Elder and Cahill 1984).
CW, Continuous wave; WBA, whole-body average; NR, not reported; AM, amplitude modulated; TSH, thyroid stimulating hormone.

In studies of effects on the HHA axis, a correlation has been observed between colonic temperatures and plasma corticosterone (Lotz and Michaelson 1978; Lu et al. 1981; Michaelson et al. 1977). An apparent threshold for corticosterone elevation occurred at SARs between 4.2 W/kg and 8.4 W/kg for a 1-hour exposure. These results were interpreted as a general, nonspecific stress reaction associated with exposure to a stressor, and not related to the "nature of the stressing agent" (Lu et al. 1981).

Toler et al. (1988) noted no differences in plasma ACTH, plasma corticosterone, plasma epinephrine, and plasma norepinephrine in cannulated rats exposed to pulsed MW. Dopamine levels were lower in the exposed animals. It was "concluded that the 435 MHz, low-level, RFR environment did not induce stress in the exposed animals when compared to the sham-exposed animals."

Levels of plasma corticosterone were not reliably affected in one study (Johnson et al. 1983) but were affected in a follow-up study. This required the use of higher MW levels or higher environmental temperatures. Significant differences were seen between all MW-exposed groups and the shams (see Fig. 3-7) (Chou et al. 1985).

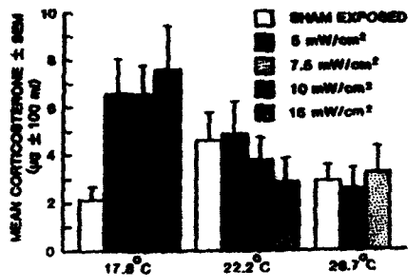

Figure 3-7. Average levels of corticosterone (± standard error of the measurement) for sham-exposed rats and animals exposed at four different values of power density and three levels of environmental temperature. From Chou et al. (1985).

Rat pups were exposed on postnatal days 1 through 6. Irradiated animals had a 1.5 to 2.5°C higher colonic temperature than controls, following exposure. On day 7, the animals were either exposed to microwaves or injected with corticotropin (ACTH), which allowed the researchers to evaluate adrenal responsiveness by measuring plasma corticosterone levels. In general, pups exposed to MW had greater adrenal responsiveness than controls, and their adrenal glands were significantly heavier. There was no difference in adrenal responsiveness in pups exposed to either MW or injected with ACTH, suggesting a stress reaction in these two groups (Guillet and Michaelson 1977).

Other researchers found no significant differences in the mass of the adrenal, thyroid, and hypophysis glands in rabbits (Guy et al. 1980; McRee et al. 1980). No differences were observed in adrenal mass in male rats (D'Andrea et al. 1979, 1986a, 1986b; Wright et al. 1984), or in weights of the anterior hypophysis, thyroid, adrenals, and testes in male and female rats (Mikolajczyk 1976). In one study, the mass of the adrenal gland was significantly elevated in MW-exposed animals. This excess was attributed to benign tumors, and "the increased adrenal weight was related to the tumors and irrelevant to the metabolic processes in the rats" (Guy et al. 1985).

Mikolajczyk (1976) evaluated influences on hormones finding no effects on follicle stimulating hormone (FSH) and growth hormone (GH), although MW-exposed rats had a significantly greater amount of LH in the hypophysis. Michaelson et al. (1977) noted that GH levels decreased with increasing power density at a given exposure duration. In cannulated rats, GH levels decreased throughout exposure, then increased to preexposure levels when exposure was terminated.

In studies with rhesus monkeys exposed to pulsed MW (width = 3 μs, PRF = 337 Hz), rectal temperatures were elevated, but there were no differences in GH and serum thyroxine (T_4) levels. Average levels of the glucocorticoid, cortisol, were significantly elevated in four of six monkeys only between 1500 and 2000 hours. This occurred at 4.1 W/kg, which increased the average rectal temperature 1.7°C (Lotz and Podgorski 1982). In a long-term study with rabbits, no significant differences were seen in cortisol (Guy et al. 1980; McRee et al. 1980).

In summary, MW radiation does influence the concentration of specific circulating hormones. The most consistent observation is an increase in adrenal cortex hormone levels. Stimulation of the adrenal cortex is important "because it points to an influence of unfavorable conditions, so called *stress stimuli*, acting upon the whole organism" (Lu, Lotz, and Michaelson 1980). Effects have also been demonstrated on luteinizing hormone, growth hormone, and thyrotropin. The lowest effective SARs were 0.21 and 2.1 W/kg, which produced a decrease in levels of the corticosterone.

A central theme in some of these experiments deals with whether the observed effects are due to a direct effect of RF on a gland, or if the effects are a nonspecific response to a stressing agent, such as heat developed as a consequence of irradiation. Although this question has not been answered completely, one group of researchers has interpreted the data as supporting "the hypothesis that the adenohypophyseal responses are the integral result of CNS processing of multiple signals from many body locations such that no single location of absorbed energy is pivotal to the onset of a response" (Lu, Lotz, and Michaelson 1980).

3.3.7 Cardiovascular, Hematologic, and Immune Effects

3.3.7.1 Cardiovascular Effects

Study measures include heart rate and arterial blood pressure. Exposure to microwave radiation has been shown to decrease (Phillips et al. 1975; Galvin and McRee 1986; Frei, Jauchem, and Heinmets 1988; Lu et al. 1992), increase (Frei, Jauchem, and Heinmets 1989; Frei et al. 1989; Lu et al. 1992), or produce no measurable effects (Toler et al. 1988) on the heart rate in test animals as shown in Table 3-12. Effects were typically transient, with the heart rate returning to normal limits after exposure (Galvin and McRee 1986; Frei, Jauchem, and Heinmets 1989; Frei et al. 1989, 1990). Findings on blood pressure include no effects (Galvin and McRee 1986; Toler et al. 1988), transient changes (Frei et al. 1989, 1990), and increases (Jauchem and Frei 1991). Differences in the outcome of these experiments could be due to differences in experimental methodologies including SAR, pulsed versus CW, orientation of the test animals relative to field vectors, and use of anesthetic.

Frei and colleagues (1989) found that increases in heart rate and arterial blood pressure were greater when rats were exposed parallel with the E field than with the H field. The E-field orientation produced greater peripheral heating, while H-field orientation produced deeper heating. A similar experiment at 5.6 GHz found no major differences between heating patterns in the E and H orientations, which may be due to the shallower penetration depth at the higher frequency (Frei et al. 1990).

Effects associated with the use of anesthesia were explored by studying unanesthetized and ketamine-anesthetized rats. In anesthetized rats, baseline arterial blood pressure and heart rate were lower, and it took significantly longer to attain a 1°C temperature rise. Heart rate was significantly elevated for both groups. Average arterial blood pressure was significantly increased in the unanesthetized state, but remained unchanged in the anesthetized state (Jauchem and Frei 1991).

In a long-term study with rats, Toler et al. (1988) noted no effects on heart rate and mean arterial blood pressure. In rabbits exposed for 6 months, there were no significant differences in the mass of the hearts (Guy et al. 1980; McRee et al. 1980). Lu and colleagues (1992) attributed changes in the heart rate of exposed rats to whole-body hyperthermia.

3.3.7.2 Hematologic Effects

Baranski (1971) observed increased WBC, bone marrow erythroblast reduction, and abnormal mitosis in erythroblast cells in guinea pigs exposed for 228 hours at 3 GHz. No effects were seen on RBC in guinea pigs and rabbits. Czerski et al. (1974) restrained and exposed rabbits head first, finding a difference in iron metabolism between animals exposed with pulsed (width = 1 μs, PRF = 1200 Hz) versus CW microwaves. Czerski also found that the extent and phase of the circadian rhythm of bone marrow stem cell mitoses was shifted for guinea pigs exposed to microwaves at different times during the day. Djordjevic, Lazarevic, and Djokovic (1977) observed no changes in hematocrit, mean cell volume, hemoglobin, total erythrocyte count, lymphocytes, and neutrophils, while total leukocyte count was not reliably affected.

Ragan et al. (1983) demonstrated no significant differences in WBC and RBC counts, while femoral marrow, hemoglobin, and plasma proteins were not reliably affected. Galvin and McRee (1986) noted no differences in leukocytes, erythrocytes, or hematocrit in rats. Smialowicz, Kinn, and Elder (1979) reported decreased WBC in 20-day neonatal rats in one experiment but not in a replicate. No changes were seen in 40-day-old rats in either experiment, and RBC, hematocrit, or hemoglobin were not affected. In another report, Smialowicz et al. (1981) found no differences in RBC count, WBC count, hemoglobin, mean cell volume of erythrocytes, hematocrit, percentage polymorphonuclear cells, and percentage lymphocytes in rats perinatally exposed.

Consistent results with mongrel dogs included decreased lymphocytes, eosinophiles, and increased neutrophils (Michaelson et al. 1964). MW exposure decreased blood volume but had no effect on hematocrit, hemoglobin, number of erythrocytes, and differential leukocytes in two female dogs. In other experiments, no consistent results were seen when dogs were exposed to pulsed MW (1285 MHz, width = 3 μs, PRF = 360 Hz) (Michaelson,

Table 3-12. Cardiovascular, Hematologic, and Immunologic Effects

Species	Frequency (MHz)	SAR (W/kg)	Average Power Density (mW/cm²)	Duration (d × min)	Effects	Reference(s)
			CARDIOVASCULAR			
Rats (male)	2450 CW	4.5 6.5 or 11.1	NR	1 × 30 1 × 30 1 × 30	No effect; bradycardia and irregular rhythm	Phillips et al. 1975
Rats (male)	2450 CW	3.7	10	1 × 360	No effect; on blood pressure or colonic temperature; *significant* (reversible) decrease in heart rate	Galvin and McRee 1986
Rats (female)	2800 CW and pulsed	8.4 12.6 16.8 21.0	30 45 60 75	1 × 180 to 1 × 240	No effect; significant decrease in heart rate for pulsed group	Frei, Jauchem, and Heinmets 1988
Rats (male)	1250 CW and pulsed (PRF = 16 Hz; 6.4 W average power)	4.75[a] (brain) 17.15 (neck)		1 × 5 or 2 × 5	No effect on mean arterial pressure and respiration; effects observed on heart rate and pulse pressure	Lu et al. 1992
Rats (female)	9300 CW and pulsed	9.3 18.6	30 60	See text	Significant transient increases in heart rate	Frei, Jauchem, and Heinmets 1989
Rats (male)	2450 CW	14.5 (E) 12.4 (H)	60 60	See text	Significant transient increases in heart rate and blood pressure; greater in E orientation	Frei et al. 1989

Table 3-12. (Continued)

Species	Frequency (MHz)	SAR (W/kg)	Average Power Density (mW/cm²)	Duration (d × min)	Effects	Reference(s)
Rats (male)	5600 CW	14 (E) 14 (H)	90 66	— —	Significant transient changes in blood pressure and heart rate	Frei et al. 1990
Rats (male)	2800 CW	14	60	—	Increased blood pressure in unanesthesized rats	Jauchem and Frei 1991
Rats (male)	435 Pulsed	0.3 to 0.35	1	168 × 1320	No differences in heart rate or blood pressure	Toler et al. 1988
			HEMATOLOGIC			
Guinea pigs and rabbits	3000 Pulsed or CW	0.7	3.5	76 × 180	Increased WBC	Baranski 1971
Rabbit	2900 CW and Pulsed 2900 CW	NR NR	3 3	37 × 120 79 × 120	Changes in iron metabolism between pulsed and CW groups; differences in circadian rhythm of stem cell mitosis	Czerski et al. 1974
Guinea pigs	2900 CW and pulsed	NR	NR	14 × 240		
Mice (female)	2880 pulsed	2.25 4.50	5 10	10 × 450 10 × 450 27 × 420 51 × 420	Increased femoral marrow cellularity; decreased volume of packed red cells, hemoglobin, and femoral marrow; increased α globulin; increased β globulin	Ragan et al. 1983

Table 3-12. (Continued)

Species	Frequency (MHz)	SAR (W/kg)	Average Power Density (mW/cm^2)	Duration (d×min)	Effects	Reference(s)
Rats (male)	2400 CW	2[b]	5	90×60	No significant hematologic effects	Djordjevic, Lazarevic, and Djokovic 1977
Rats (male)	2450 CW	3.7	10	1×360	No hematologic effects	Galvin and McRee 1986
Rat pups	2450 CW	0.7 to 4.7	5	37×240 57×240	No consistent differences	Smialowicz, Kinn, and Elder 1979
Rat pups	100 CW	2.5 to 3	46	42×240	No differences	Smialowicz et al. 1981
Dogs	2800 Pulsed	8[b]	165	1×120 1×180	Decreased lymphocytes; increased neutrophils, decreased eosinophiles; increased total leukocytes, decreased lymphocytes and eosinophiles; increased total leukocytes and neutrophiles, decreased eosinophiles	Michaelson et al. 1964
		5[b]	100	1×360		
	1285 Pulsed	4[b]	100	1×360		
	200 CW	30[b]	165	1×360		
Dogs	24,000 Pulsed	NR	24	600×990	Decreased blood volume; no consistent effects	Michaelson, Howland, and Deichmann 1971
	1285 Pulsed	1, 2.5,[b] 5	20, 50 100	1×360 10×360 20×360		
Mice	34,000	NR	0.02	10×1020	Decreased leukocytes and granulocytes	Rotkovska et al. 1993

Table 3-12. (Continued)

Species	Frequency (MHz)	SAR (W/kg)	Average Power Density (mW/cm^2)	Duration (d×min)	Effects	Reference(s)
Monkeys	28	0.007[b]	25	24×1380	No hematologic effects	Wright et al. 1984
Rats (female)	0.018 Pulsed	NR	See text	37×420	Reduced RBC, WBC, and lymphocytes in highest exposure group	Stuchly et al. 1988
Rabbits	2450 CW	1.2 to 2.2	7 to 10	40×480 to 85×480	Significant differences in RBC	Ferri and Hagan 1977
Rabbits	2450 CW	1.5	7	180×1380	Significant changes in eosinophils, serum albumin/total globulin ratio	McRee et al. 1980
Rats (male)	2450 Pulsed	0.15 to 0.4	0.48	750×1260	Reduced eosinophils, and neutrophils	Kunz et al. 1984
Rats (male)	2450 Pulsed	0.15 to 0.4	0.48	180×1260 360×1260	No differences in hematology, reduced γ globulin	Chou et al. 1985
Rats (male)	2450 CW	1 to 1.5	5	80×480	Variable differences in RBC, WBC, and total plasma sulfhydryls	D'Andrea et al. 1979
Rat (male)	2450 CW	0.14	0.5	90×420	No differences found	D'Andrea et al. 1986a
Rat (male)	2450 CW	0.7	2.5	98×420	No differences found	D'Andrea et al. 1986b
Rats (male)	435 Pulsed	0.3 to 0.35	1	168×1320	No differences in RBC, WBC, eosinophils, and neutrophils	Toler et al. 1988

Table 3-12. (Continued)

Species	Frequency (MHz)	SAR (W/kg)	Average Power Density (mW/cm^2)	Duration (d×min)	Effects	Reference(s)
Rabbit RBC[c]	2450 3000 3950	86 22, 131 110	58 10, 58 58	1×20 or 1×180	No effects on RBC permeability and osmotic fragility	Liu, Nickless, and Cleary 1979
Rabbit, human, dog RBC[c]	3000	47, 136, 173, 200	10, 29, 36, 42	1×180		
Rabbit[c] RBC	10	NR	630, 900 V/m	1×120	No effects	Cleary, Liu, and Garber 1985a
	50	NR	100, 460 900 V/m	1×120	No effects; hemolysis	
	100	NR	100, 450 900 V/m	1×120	No effects; hemolysis	
Rabbit RBC[c]	3000	NR	1, 5, or 10	1×15 1×30 1×60 1×120 1×180	Enhanced K^+ and hemoglobin leakage	Baranski, Szmigielski, and Moneta 1974
Rabbit[c] or human RBC	2450	NR	10	1×45	No differences in K^+ or hemoglobin	Peterson, Partlow, and Gandhi 1979
			IMMUNOLOGIC			
Mice (male)	2450 CW	14 average	NR	1×30 3×30	Weak stimulatory effect on B cells but not T cells; increase in CR^+ B cells	Wiktor-Jedrezjczak et al. 1977
Mice	2450 CW	See text	NR	1×20	Defined threshold for increase in CR^+ B cells	Sulek et al. 1980
Mice	2450 CW	10 to 14	NR	1×20	Demonstrated genetic control of increase in CR^+ B cells	Schlagel and Ahmed 1982

Table 3-12. (Continued)

Species	Frequency (MHz)	SAR (W/kg)	Average Power Density (mW/cm^2)	Duration (d×min)	Effects	Reference(s)
Rats (male)	2450 Pulsed	0.15 to 0.4	0.48	390×1260	Increased B and T lymphocytes and enhanced lymphocyte response to mitogen stimulation; no differences	Kunz et al. 1983
				750×1260		
Rat (male)	2450 Pulsed	0.15 to 0.4	0.48	180×1260	Increased marrow hematopoietic progenitor cells, increased proliferative responses of splenic B cells, decreased B cells in marrow; increased hematopoietic precursors, decreased avg. cell surface density of sIg	Chou et al. 1985
				360×1260		
Mice (female)	2450 CW	11	15	1×30 to 17×30	Enhanced/ reduced mitogen responsiveness	Huang and Mold 1980
Mice (male)	26 CW	5.6	800	1×15 or 20×15	Reduced lymphocytes and increased neutrophils, increased T and B cells; no effects	Liburdy 1979
		0.36				
Hamsters	2450 CW	13	25	1×60	Enhanced viricidal activity of macrophages	Rama Rao, Cain, and Tompkins 1984

Table 3-12. (Continued)

Species	Frequency (MHz)	SAR (W/kg)	Average Power Density (mW/cm²)	Duration (d×min)	Effects	Reference(s)
Mice	3000 CW	NR	40	4×120 to 14×120	Virus inhibition due to hyperthermia	Szmigielski et al. 1977
Hamsters	2450 CW	8, 13	15, 25	1×60	Increased antibody response	Rama Rao, Cain, and Tompkins 1985
Mice (male)	9400 Pulsed	0.015	0.03	5×600	No change; increased or decreased antibody response as function of AM frequency	Veyret et al. 1991
Mice (male)	2950 Pulsed	0.5[d]	0.5	36×120 72×120	Increased antibody producing cells; no effect	Czerski 1975
Mice (female)	2880 Pulsed	4.50	10	20×180	No consistent effects	Ragan et al. 1983
Mice	2450 CW	2 to 3 6 to 8	5 15	72×120	Decreased antineoplastic resistance	Szmigielski et al. 1982
Rat pups	2450 CW	0.7 to 4.7	5	37×240 57×240	No differences; increased response of lymphocytes to mitogen stimulation	Smialowicz, Kinn, and Elder 1979
Rat pups	100 CW	2.5 to 3	46	22×240 42×240	No differences in response to mitogen stimulation	Smialowicz et al. 1981

Table 3-12. (Continued)

Species	Frequency (MHz)	SAR (W/kg)	Average Power Density (mW/cm²)	Duration (d×min)	Effects	Reference(s)
Mice (male)	2450 CW	11, 14, 22, 29	15, 20, 30, 40	1×30	Significant increase in CR^+ spleen cells in 16-week-old mice at highest dose rate	Smialowicz, Brugnolotti, and Riddle 1981
Rat lymphocyte[c]	2450	0.7, 1.4 2.8	5, 10, 20	1×240 1×1440 1×2640	No change to lymphocytes to mitogen stimulation	Hamrick and Fox 1977
Rabbit PMN[c]	100 CW or AM: 20 Hz	120 to 341	250 to 410 V/m	1×30 1×60	No effects	Cleary, Liu, and Garber 1985b
Rat lymphocytes[c]	450 450 AM: 3, 16, 40, 60, 80, and 100 Hz	NR	1.5	1×240	No change; suppressed T-lymphocyte activity	Lyle et al. 1983
Human leukocyte[c]	2450	0.5 to 4[e]	NR	1×120	No effects	Roberts, Lu, and Michaelson 1983
Human leukocyte[c]	2450 Pulsed 16 or 60 Hz	0.29 to 4[e]	NR	1×120	No effects	Roberts, Michaelson, and Lu 1984
Human lymphocytes[c]	450 450 AM: 16, 40, and 60 Hz	NR	1[d]	1×30	No change in enzyme activity; reduced enzyme activity	Byus et al. 1984

[a]SAR reported per watt of transmitted power into the brain or neck.
[b]SAR estimated from Durney, Massoudi, and Iskander (1986).
[c]In vitro study.
[d]Peak intensity.
[e]According to Budd and Czerski (1985), Roberts and colleagues used an average SAR to represent exposure, although "the SAR at the location of the cells within the sample was about twice the average SAR."
NR, not reported; AM, amplitude modulated; CW, continuous wave; RBC, red blood cell; WBC, white blood cell; CR, complement receptor; PMN, polymorphonuclear leukocytes.

Thomson, and Howland 1965; Michaelson, Howland, and Deichmann 1971). No effects were noticed in six cynomolgus monkeys on full blood and platelet counts, peripheral blood smear, iliac crest marrow smear, platelet aggregation, serum B12, red cell folate assays, reticulocyte count, and 12 biochemical measures (Wright et al. 1984). Rotkovska and colleagues (1993) reported that hairless mice exposed to millimeter waves at very low levels of power density, 20 $\mu W/cm^2$, experienced decreases in leukocyte count and percentage of granulocytes. This was attributed to a stress reaction mediated through skin receptors.

Female Sprague-Dawley rats were exposed prior to and during pregnancy at flux densities of 0, 5.7, 23 and 66 μT. In the 66-μT group, RBC and WBC were significantly reduced, while mean corpuscular hemoglobin was significantly reduced in the 23-μT group. Unaffected measures included hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, platelets, and bone marrow (Stuchly et al. 1988).

As noted in Section 3.3.1, a number of long-term studies have demonstrated hematologic effects. Rabbits had statistically significant differences in RBC count but not in WBC count (Ferri and Hagan 1977). In another study with rabbits, the WBC count was nonsignificantly reduced in the MW-treated animals immediately after exposure. A marginally significant ($P = 0.046$) increase was found in serum albumin, and there was a significant decrease in eosinophils. Thirty days after exposure, there were no differences in either measure. It is difficult to establish the biologic relevance of these findings since 6 of 7 serum protein measures and 12 of 13 hematologic parameters were not affected at the termination of exposure (Guy et al. 1980; McRee et al. 1980).

In a long-term study with rats, eosinophils and neutrophils were not reliably affected, and no differences were seen in RBC and WBC counts, hematocrit, hemoglobin, serum albumin, and globulin (Kunz et al. 1984). Using a similar protocol, no significant differences were found for 11 hematologic measures and a large number of serum chemistry parameters. Electrophoresis of serum proteins showed one of five measures had a marginally significant ($P = 0.0477$) reduction at 6 months (γ globulin), but no measures were significantly affected at 12 months (Chou et al. 1985). In rats, levels of RBC, WBC, and total plasma sulfhydryls were not reliably affected, and there were no differences in hemoglobin, hematocrit, polymorphic neutrophils, and lymphocytes (D'Andrea et al. 1979). In similar studies, no significant differences were found in blood levels of cholinesterase and sulfhydryl groups (D'Andrea et al. 1986a, 1986b). A study of pulsed (width = 1 μs, PRF = 1 kHz) 435-MHz microwaves on cannulated rats showed no differences in hematocrit; monocytes; heart rate; mean arterial blood pressure; and counts of RBC, WBC, neutrophils, and eosinophils (Bonasera, Toler, and Popovic 1988; Toler et al. 1988).

Selected findings on in vitro effects on various cell types are reported next. An in-depth review has been performed by Cleary (1989). Erythrocyte permeability and osmotic fragility were not affected by MW radiation (Liu, Nickless, and Cleary 1979), while erythrocyte hemolysis appears to have an E-field strength dependence (Cleary, Liu, and Garber 1985a). Baranski, Szmigielski, and Moneta (1974) observed enhanced leakage of potassium (K^+) and hemoglobin in rabbit RBC. Both measures increased with increasing power density at a given exposure duration and with increasing exposure duration at a given power density. In an attempt to replicate this finding, Peterson, Partlow, and Gandhi (1979) evaluated the RBC membrane while monitoring RBC temperature. Enhanced leakage of hemoglobin and K^+ was found to depend on the rate of heating and the magnitude of the temperature increase. A dosimetric comparison was made of a stationary sample holder and a rotating sample holder. A highly asymmetric SAR distribution was found in the stationary holder but not in a rotating holder. The difference in these results and those of Baranski, Szmigielski, and Moneta (1974) are attributed to irradiation technique.

In summary, acute and chronic studies using CW and pulsed RF fields have been performed on rats, dogs, guinea pigs, rabbits, and monkeys. Dependent upon the study design, effects have been observed on RBCs, WBCs, granulocytes, and serum proteins, although these have not been established consistently. For example, experiments with 2.8- to 3-GHz pulsed microwaves showed no consistent effects with SARs of 0.7 to 8 W/kg, although WBCs were affected at the lowest SAR (Baranski 1971; Ragan et al. 1983; Michaelson et al. 1964). In vivo studies have been performed with CW, 2450-MHz microwaves with SARs from 0.17 to 4.7 W/kg (Djordjevic, Lazarevic, and Djokovic 1977; Galvin and McRee 1986; Smialowicz, Kinn, and Elder 1979; Ferri and Hagan 1976; McRee et al. 1980; Kunz et al. 1984; Chou et al. 1985; D'Andrea et al 1979, 1986a, 1986b). Again, no end point was reliably affected (see Table 3-12). In total, the results have been equivocal and no trends have been observed. Also, some of the effects were shown to be reversible when the exposure was ceased.

3.3.7.3 *Immunologic Effects*

A large number of in vivo and in vitro experiments have examined effects on immune function. The reports selected for inclusion here represent the variety of findings. For the interested reader, detailed reviews of immunologic effects are available (Roberts 1983; Elder and Cahill 1984; Budd and Czerski 1985; Smialowicz 1987).

3.3.7.3.1 In Vivo Effects Many experiments have examined responsiveness of T and B lymphocytes. An example of a representative protocol for such an experiment includes sacrificing the animals, removing the spleens, and extracting splenic cells, subsequent to exposure. These cells are cultured in either a growth media plus tritiated thymidine (^{3}H-thymidine) or in the media plus ^{3}H-thymidine in the presence of T- or B-cell mitogens (substances that stimulate lymphocytes to proliferate). Following incubation, a responsiveness or stimulation index is calculated as the ratio of the counts per minute of the mitogen-stimulated group to the counts per minute of the growth-media group. The indices for the RF-exposed and sham-exposed groups are then analyzed statistically.

Wiktor-Jedrzejczak et al. (1977) observed a significant increase in the number of complement receptor positive (CR^+) B cells for restrained mice exposed head first in a waveguide. Sulek et al. (1980) found that a single 20-minute exposure stimulated a significant increase in CR^+ B cells in mice. An apparent threshold was observed, where CR^+ cells increased at SARs > 5 W/kg (SA > 9 to 10 J/kg). In a long-term study, there was a significant stimulatory effect on T and B lymphocytes in rats at 13 months but not at 25 months (Kunz et al. 1983). Mitogen stimulation of lymphocytes was not demonstrated in a follow-up study, where effects were found in 7 of 70 immune parameters evaluated (see Table 3-12). Even if the null hypothesis of no difference between MW- and sham-exposed animals were true, at the 0.05 level of significance, 3.5 significant effects would have been expected (Kunz et al. 1985).

Wiktor-Jedrzejczak et al. (1977) found that splenic B cells from exposed mice exhibited an increased responsiveness to mitogens after a single exposure, but no changes were found with T cells. Huang and Mold (1980) reported variable mitogen responsiveness in Balb/c mice. No effects were found on the cytotoxic activity of lymphocytes against an inoculation of leukemic cells. The outcome of another experiment indicated that peritoneal macrophages may be activated by MW exposure. Liburdy (1979) elevated the core temperature in mice 2 to 3°C by exposure to RF or warm air. Both treatments reduced lymphocytes and increased neutrophils, but the change was more robust for the RF exposure.

Normal control, sham-exposed, and MW-exposed hamsters were injected with a lethal dose of vesicular stomatitis virus 1 day after MW exposure. MW exposure raised core-body temperature 2.5 to 3°C, to a maximum of 40.5°C. The mean survival times for MW-exposed animals were significantly longer, where 25 percent of the high-dose

group and 33 percent of the low-dose group survived, while all of the sham-exposed and normal-control animals died. Survival of the MW-exposed animals was attributed to activation of peritoneal macrophages, which increased the resistance to viral infection (Rama Rao, Cain, and Tompkins 1984). Szmigielski and colleagues (1977) found a significant decrease in mortality rate and tail lesions in mice receiving MW hyperthermia immediately following infection with either herpes simplex virus or vaccinia virus. The results of these studies support the hypothesis that the observed effects have a thermal basis.

Rama Rao, Cain, and Tompkins (1985) noted an increase in antibody response in hamsters immunized with sheep red blood cells (SRBC, a T-cell-dependent antigen). Antibody responsiveness with SRBC was nonsignificantly elevated for mice exposed with pulse modulated waves (width = 1 μs, PRF = 1 kHz). When the fields were amplitude modulated, the response was dependent upon the modulation frequency. A similar, complex response pattern was observed in an experiment with glutaric-anhydride conjugated bovine serum albumin (GA-BSA) (Veyret et al. 1991). Czerski (1975) found an increase in antibody-producing cells in the lymph nodes in animals immunized with SRBC following exposure (width = 1 μs, PRF = 1200 Hz) for 6 weeks but not for 12 weeks. According to Roberts (1983), these results need to be interpreted with caution because the controls were not sham-exposed, and there was no statistical analysis of the data.

A skin challenge test in mice was affected with pulsed microwaves (width = 2.3 μs, PRF = 100 Hz) and injections of keyhole limpet hemocyanin or skin painting with dinitrofluorobenzene (DNFB). Skin thickness of DNFB-exposed mice was significantly less, but this was not observed in a replicate experiment. No significant differences were seen in response to T- and B-cell mitogens (Ragan et al. 1983).

A decrease in the natural antineoplastic resistance in Balb/c mice has been reported. Animals received intravenous injections of neoplastic L_1 sarcoma cells, then were irradiated for 1 to 3 months as shown in Table 3-12. A special control group was maintained in small cages to induce chronic-stress confinement. A statistically significant increase in lung cancer nodules was found for the high-SAR group and the confined group after 1 month, and in both MW-exposed groups and the confined group after 3 months of exposure (Szmigielski et al. 1982).

In a study of immune effects on young animals, cultured lymphocytes from rats exposed from day 6 of gestation to 40 to 41 days of life had an increased response to mitogen stimulation. An increased response was not seen in 20- to 21-day-old rats (Smialowicz, Kinn, and Elder 1979). Smialowicz et al. (1981) reported no differences in the mitogen-stimulated response of lymphocytes from the lymph nodes and the blood in rat pups. In another experiment, Smialowicz, Brugnolotti, and Riddle (1981) observed no changes in CR^+ spleen cells in 10- to 12-week-old mice 6 days after a single 30-minute exposure but noted significant differences in 16-week-old mice exposed at 29 W/kg. Although body temperature was not monitored, the authors report that at the highest SAR mice demonstrated that they were under thermal stress by coating their fur with saliva or urine in an attempt to cool themselves.

3.3.7.3.2 In Vitro Effects No significant differences were found in mitogen-treated rat lymphocytes exposed to microwaves (Hamrick and Fox 1977). High-level SARs produced no effects on viability or phagocytic ability of rabbit neutrophils exposed to CW or amplitude-modulated fields (modulation depth = 95 percent) (Cleary, Liu, and Garber 1985b). Cytotoxic activity of rat T lymphocytes was suppressed in an experiment using an AM, 450-MHz RF field. The most effective modulating frequency was 60 Hz, while the least effective was 3 Hz. No effects were observed with just the 450-MHz carrier wave, and the observed effects were reversible (Lyle et al. 1983). Roberts, Michaelson, and Lu (1984) and Roberts, Lu, and Michaelson (1983) reported no differences in mitogen-stimulated responses or in DNA and protein synthesis.

Byus et al. (1984) exposed cultured human tonsil lymphocytes (50 percent B cells, 50 percent T cells) to a 450-MHz carrier or a sinusoidally AM (3 to 100 Hz; modulation depth = 75 to 85%) carrier. Extracts from lymphocyte cells were assayed for the activity of protein kinase enzymes. No differences were observed in the activity of cyclic-AMP-dependent protein kinases at an AM frequency of 16 Hz. No change was seen in cAMP-independent histone kinase activity when cells were exposed at 450 MHz or to the carrier modulated at 3, 6, 80, and 100 MHz. However, cAMP-independent histone kinase activity was reduced at 16, 40, and 60 Hz, with the maximum response at 16 Hz. The observed reduction was transient at AM frequencies of 16 and 60 Hz. The authors attribute the frequency-dependency to a windowed response.

3.3.7.3.3 Conclusions Experiments have shown that the immune system may be a sensitive indicator of RF-induced biologic effects. The lowest SARs that are biologically effective in test animals in a relatively consistent manner are in the range of 0.15 up to 0.7 W/kg. Clearly, SARs between 1 and 10 W/kg can produce immune effects; however, no clear response pattern has been observed, and the results from some studies suggest that observed effects are transient. MW exposure prior to mitogen stimulation produced variable effects on lymphocytes, including stimulatory and inhibitory effects, and no change. Some studies showed an increase in antibody response after antigenic immunization, but a variable response was seen in others. In some studies, effects appear to depend upon the modulation frequency.

3.3.8 Genetic Effects

Czerska et al. (1992) found that temperature plays a significant role in lymphoblastoid transformation of human cells exposed to conventional heat or CW microwaves. Results with pulsed (width = 1 μs; PRF 100 to 1000 Hz; also, see Table 3-13) MW showed significant differences in the numbers of lymphoblastoid cells under nonheating conditions, compared with cells heated conventionally and by CW MW. Hence, pulsed and CW microwaves acted differently in this experiment, although mechanisms supporting such an interaction are not known.

Krause et al. (1991) found no differences in growth and survival of cells exposed at SARs of 130 and 1300 W/kg. The effects on the expression of two interferon-regulated enzymes were differential. Specific activity of 2-5A synthetase was unaffected, while changes were observed in RNase L. The changes did not appear to be detrimental to the cell, as measured by postexposure viability, plating efficiency, or proliferation rate. Saffer and Profenno (1992) observed a frequency-independent increase in β-galactosidase expression in microwave-exposed cells. They speculate that "small thermal gradients" may produce the effects. Das and colleagues (1991) found that low-level microwave radiation significantly increased neuron specific enolase activity.

Sulek et al. (1980) observed that some strains of mice were susceptible to MW-induced increases in CR^+ cells, while other strains were nonresponders. This discovery led researchers to suggest that the microwave-induced reversible increase in CR^+ was genetically controlled. In a study designed to examine this hypothesis, the results showed that there was a genetic basis for increased CR-bearing B lymphocytes, and this control was effected by a single regulatory gene (Schlagel and Ahmed 1982).

Garaj-Vrhovac, Fucic, and Horvat (1992) exposed human lymphocytes at a constant temperature of 22°C. SARs were not determined. Subsequent to exposure, cells were stimulated with the mitogen, phytohemagglutinin, then fixed for chromosomal analysis. Statistically significant differences between controls and exposed cells were observed for 30- and 60-minute exposures at 30 mW/cm^2. Significant increases in all types of observed aberrations (pooled data) occurred for exposures at 10 and 30 mW/cm^2. The data suggest a dose-dependent increase in the aberration rate. Maes and colleagues (1993) observed an increase in the frequency of structural chromosome aberrations and micronuclei under isothermal test conditions (36.1°C), which they interpreted as consistent with the

findings of Garaj-Vrohovac and colleagues. No effects were seen in cell kinetics and sister chromatid exchange frequency.

In summary, in the study by Czerska et al. (1992), CW and pulsed exposures resulted in the same temperature rise and energy absorption. However, the rate of the temperature change was considerably higher in cells experiencing pulsed exposures, raising the possibility of thermoacoustic effects in these cells, even at nonheating exposure levels. This could explain the differences observed between CW and pulsed exposures.

The study by Das et al. (1991) had insufficient information to allow an adequate review, since only an abstract from a poster session was published. Garaj-Vrhovac, Fucic, and Horvat (1992) report statistically significant differences in aberrations, and their data are suggestive of a dose–response effect with pooled data. When analyzed by specific type of aberration, the data do not show significant differences between exposed and control cells, nor do they demonstrate a dose–response relationship. The authors exposed the cells under isothermal conditions (22°C) but provide insufficient information for an assessment of their methods. This study needs replication in an independent laboratory, as does the study by Maes and coworkers (1993).

In conclusion, these genetic studies produced some positive findings that require replication by an independent laboratory. There are a number of *unanswered* methodologic questions in some of the studies.

3.3.9 Cancer

Included in this section is a small number of studies in test animals and of the potential for enhanced cell proliferation in in vitro studies. Few studies have actually been designed to assess the potential promotional effects of RF fields. Generally, the conclusions that can be drawn from the reviewed studies are limited because most studies use a single sex of one species at 1 SAR. Some studies were designed to evaluate end points other than cancer, and their methodology does not provide a rigorous statistical or histopathologic evaluation of the cancer results. These results are compiled in Table 3-13. Reviews of RF exposure and cancer are available for the interested reader (Kirk 1984; Adey 1988; Szmigielski and Gil 1989).

3.3.9.1 In Vivo Studies

Prausnitz and Susskind (1962) exposed male mice (see Section 3.3.1 for details) finding that 10% of the control animals and 35% of the MW-exposed mice that died during the experiment had cancer of the white cells. No differences were found in mice sacrificed at 7 months, but 30% of the exposed and 10% of the controls had leukosis at 16 months. At 19 months, evidence of abdominal lymphoma was found in 18% of the exposed and 21% of the control animals. In commenting on this study, Kirk (1984) devised a statistic to test the prevalence rates in the MW-treated and controls, with a finding of marginal nonsignificance ($P \sim 0.06$). Kirk notes that the results from this study were difficult to interpret because of "problems with the biological protocol, the lack of sound statistical methodology in experimental design and data analyses, and the questionable significance of what was reported."

Two experiments were designed to study the effect of increasing the metabolic rate on longevity in CFW mice. Four pregnant mice were exposed, and the pups (selected from exposed and control groups) were injected with an homogenate of a lymphoreticular cell sarcoma and the avian, fast reticuloendothelial T virus on postnatal day 16. Animals were sacrificed on postpartum day 93. The incidence of tumors in mice irradiated in utero or in utero plus postnatally was significantly lower than the incidence in mice not exposed in utero. The second experiment used more mice, with an initial finding that the percentage of MW-treated mice with tumors was less than the sham-exposed group. At 4.5 months there were no differences between the groups, and the percentage of exposed animals with tumors finally exceeded the numbers for the sham-exposed group: "It is evident that microwave-induced hyperthermia in utero did not significantly alter the absolute incidence of tumors

Table 3-13. Genetic Effects and Cancer Studies

Species	Frequency (MHz)	SAR (W/kg)	Average Power Density (mW/cm^2)	Duration (d × min)	Effects	References
			GENETIC EFFECTS			
Human lymphocytes[a]	2450	0.8 to 12.3	NR	5 × 1440	Enhanced lymphoidblastoid transformation by conventional heating, CW, and pulsed MW; pulsed MW increased transformation at nonheating levels (37°C)	Czerska et al. 1992
Murine L929 cells[a]	2450 CW	130	96	1 × 240	Increase in specific activity of RNase L; no effects to 2-5A synthetase	Krause et al. 1991
E. coli[a]	2550	10	NR	1 × 270	Increased in activity of a marker gene	Saffer and Profenno 1992
Mouse neuroblastoma and rat glioma cells[a]	915 AM: 16 Hz	0.5	NR	1 × 30	Significant increase in neuron specific enolase	Das et al. 1991
Human lymphocytes[a]	7700	NR	0.5, 10, 30	1 × 10 1 × 30 1 × 60	Dose-dependent increase in micronuclei and total aberrations	Garaj-Vrhovac, Fucic, and Horvat 1992
Human lymphocytes[a]	2450 Pulsed (PRF = 50 Hz)	75[b]	240 V/m[b]	1 × 30 or 1 × 120	Increase in chromosome aberrations for 120-minute exposures	Maes et al. 1993
			CANCER STUDIES			
Mice (male)	9200 Pulsed	50[c]	100	295 × 4.5	Leukosis or leukemia in exposed mice	Prausnitz and Susskind 1962

Table 3-13. (Continued)

Species	Frequency (MHz)	SAR (W/kg)	Average Power Density (mW/cm^2)	Duration (d×min)	Effects	Reference(s)
Mice	2450 (60 Hz, sinusoidally modulated)	35	NR	4 × 20 40 × 20 36 × 20 4 × 20	Lower incidence of tumors in mice with in utero MW treatment; no difference with sham-exposed group; no difference in absolute incidence of tumors	Preskorn, Edwards, and Justesen 1978
Mice	2450 CW	2 to 3[d] 6 to 8	5 15	30 × 120 60 × 120 90 × 120	Increase in neoplastic nodules; increase in spontaneous breast cancer; accelerated skin cancer	Szmigielski et al. 1982
Mice (female)				60 × 120 to 356 × 120		
Mice				30 × 120 90 × 120 or 150 × 120		
Mice	2450	2, 4, 6[d]	5, 10, 15	30 × 120 60 × 120 90 × 120 180 × 120	Shortened skin cancer development times	Szudzinski et al. 1982
Mice (female)	2450 CW and pulsed	1.2	1	< 276 × 150	No difference in tumor development, survival time	Santini et al. 1988
Rats (male)	2450 Pulsed	0.15 to 0.4	0.48	750 × 1260	Higher incidence of malignant tumors from collapsed data; elevated adrenal mass	Guy et al. 1985 Johnson et al. 1984

Table 3-13. (Continued)

Species	Frequency (MHz)	SAR (W/kg)	Average Power Density (mW/cm^2)	Duration (d×min)	Effects	References
Rats (female)	0.002 CW	NR	2 mT	9 × 60	No difference in tumor weight	Baumann et al. 1989
C3H/10T½[a] cells	2450 Pulsed	0.1, 1, 4.4	NR	1 × 1440	Increase in neoplastic transformations	Balcer-Kubiczek and Harrison 1985, 1989, 1991
LN71[a] glioma cells	2450 or 27 CW	0 to 50 50 to 200	< 200 V/m 150 to 350 V/m (estimated)	1 × 120	Increased thymidine and uridine incorporation; suppressed incorporation	Cleary, Liu, and Merchant 1990
H35[a] heptoma cells;[a] CHO; 294T melanoma cells	450 AM (see text)	NR	1.0	1 × 60	Modulation frequency dependent changes in ODC activity	Byus et al. 1988
Murine L929 cell[a]	915 AM (see text)	2.5	70 V/m	1 × 480	Enhancement of ODC activity when the carrier was modulated	Litovitz et al. 1993

[a] In vitro study.
[b] SAR not determined by irradiation but calculated by passing a DC electric current through a resistor located in the sample. E-field calculated from the absorbed power and conductivity.
[c] Estimate based on Durney, Massoudi, and Iskander (1986).
[d] Gang exposure techniques (10 animals per cage) were used during actual exposures, which raises questions about the applicability of the estimated SARs, which were determined for a single mouse cadaver.
CW, Continuous wave; NR, not reported; mT, millitesla, unit of magnetic flux density; AM, amplitude modulated; ODC, ornithine decarboxylase.

but only delayed the genesis of a palpable neoplasm" (Preskorn, Edwards, and Justesen 1978). Animals that received in utero irradiation lived longer than controls, regardless of whether they had tumors or not. The authors speculate that the observed effects are due to "an enhanced immunocompetency that has its origins in elevation of fetal—and, perhaps, of maternal—temperature" (Preskorn, Edwards, and Justesen 1978).

Szmigielski et al. (1982) evaluated the potential for 2450-MHz MWs to accelerate de-

velopment of induced and spontaneous tumors in mice exposed in the far field. For the long-term trials, 10 animals were housed, caged, and exposed for 2 h/d, 6 d/wk. Three bioassays were performed: lung cancer colony, spontaneous breast tumors, and benzopyrene-induced skin cancer. The lung cancer colony assay involved the intravenous injection of neoplastic cells into Balb/c mice. Animals were sacrificed after 1, 2, or 3 months and evaluated for neoplastic nodules. Female C3H/HeA mice, which have a high incidence of spontaneous breast cancer, were exposed from around 1.5 to 12 months of age. Evaluations were performed every two weeks, and the cancer development time for 50% of the animals (CDT_{50}) and the mean survival time for 50% (MST_{50}) were determined. In the skin cancer study, mice were depilated and painted with 0.01 mL of 5% 3,4-benzopyrene (B(a)P) in a 9:1 acetone-benzene solvent. Controls were treated with just the solvent. MW treatment was delivered either 1 or 3 months before B(a)P treatment or concurrently and extending for 5 months. Sham-irradiated controls and chronic-stress controls were used for all three bioassays. This latter group was composed of male Balb/c mice that received no MW exposure but were maintained in substantially smaller cages. Mice exposed at 6 to 8 W/kg or chronically confined had significantly elevated numbers of lung nodules after 1 or 3 months of exposure. After 3 months, the 2- to 3-W/kg group was also significantly higher than controls but still lower than the chronic confinement group. CDT_{50} and MST_{50} for spontaneous breast cancer were significantly shorter for both MW-exposed and the confined groups. CDT_{50} was 219 days in the 6- to 8-W/kg group, 255 days for chronic confinement, 261 days for 2 to 3 W/kg, and 322 days for controls. Time to develop B(a)P-induced skin cancer was significantly accelerated in animals exposed to microwaves or confined, and survival time was shorter.

In another study, these researchers examined the co-carcinogenic effects of microwave radiation in regard to B(a)P-induced skin cancer in male Balb/c mice. Dosimetric evaluations were performed with cadavers. In two groups of mice (2 and 6 W/kg for 6 months), microwave radiation and B(a)P were applied simultaneously. The mean CDT and CDT_{50} were statistically significantly different for the 6-W/kg group compared with sham-controls. The MST of the control animals was longer than that for animals exposed at both 2 and 6 W/kg. In three groups, animals were irradiated at 4 W/kg for 1, 2, or 3 months prior to inception of B(a)P application. CDT was significantly shortened in the MW-exposed groups but was most pronounced in the group exposed for 3 months prior to skin painting. These results suggest that MW radiation may be co-carcinogenic, since it was applied prior to or simultaneously with the initiating carcinogen, B(a)P. In terms of a gross mechanism, the authors report finding no evidence that MW exhibited a direct carcinogenic effect. On the other hand, the stimulatory effects observed could be due to a thermal response. The authors claim the MW doses were nonthermal, but the reported study design does not include supportive data from core-body temperature measurements in live animals, just conclusions from exposure of the cadavers (Szudzinski et al. 1982).

Santini and colleagues (1988) studied potential effects of 2450-MHz microwaves to accelerate development of B16 melanoma in C57BL/6J mice. Mice were exposed in the far field within an anechoic chamber until the animals died (up to 690 hours of exposure). Prior to MW exposure melanoma cells were subcutaneously implanted in the mice. There were no differences in tumor development, the numbers of surviving and dead animals, and survival times.

In a lifetime study, male rats were exposed at SARs from 0.4 to 0.15 W/kg and evaluated as indicated in Section 3.3.1. Gross pathologic and histopathologic evaluations were performed on rats when they died spontaneously or when they were sacrificed (Guy et al. 1985). An organ-mass analysis indicated that the mass of the adrenal glands at the final kill (25 months) was significantly increased in MW-exposed rats. The increase was attributed to tumor growth (Johnson et al. 1984). A total of

12 nonneoplastic lesions of all organs and tissues were found. The numbers of benign tumors were not different for MW-exposed and sham controls. One hundred ninety-two neoplastic lesions were identified, with the endocrine system having the "highest incidence of neoplasia in the aging rats, as is to be expected in this experimental animal" (Guy et al. 1985). The neoplasms occurred in 45 exposed and 40 sham-exposed animals. Malignant neoplastic lesions were subdivided into primary and metastatic. The numbers of metastatic lesions were too low to allow a meaningful analysis. No single type of primary malignancy was significantly increased, but when the data for primary malignant lesions at death were combined for all organs and tissues, the exposed animals had a significantly increased (approximately fourfold) incidence (Johnson et al. 1984; Guy et al. 1985; Chou et al. 1992).

Female Wistar-Furth rats received an implantation of mammary adenocarcinoma near the lower nipples. Animals were exposed in a restrained condition in four replicate experiments. In the first two, animals were treated with either 0.1-, 1-, or 2-mT B fields. Only the highest flux density was used in the last two trials. SARs were not reported, because it is not a meaningful concept at lower frequencies. There were no significant differences in tumor weight, but in most instances exposure at 2 mT appeared to have a nonsignificant inhibitory effect on tumor development (Baumann et al. 1989).

3.3.9.2 *In Vitro Studies*

Balcer-Kubiczek and Harrison (1985, 1989, 1991) evaluated the neoplastic transformation of C3H/10T½ mouse cells when exposed to pulse modulated (120 Hz), 2450-MHz microwaves, and a tumor promoter, TPA (12-O-tetradecanoylphorbol-13-acetate), a phorbol ester.

In one study, cells were exposed to x-rays or B(a)P, or a combination of MW plus these initiating agents. Following x-radiation, cells were plated onto medium containing TPA dissolved in dimethylsulfoxide (DMSO) or just the medium plus DMSO. Postexposure evaluations were performed for cell survival and induced neoplastic transformation. The transformation rate was not affected by exposure to MW with either B(a)P or x-rays in the absence of the promotor TPA. The transformation rate was significantly enhanced by treatment with either x-rays + TPA or x-rays + MW + TPA. This lead to the conclusion that microwave radiation might induce "latent transformation damage which can then be revealed by the action of tumor promotors" (Balcer-Kubiczek and Harrison 1985).

In a later experiment, cells were either irradiated with just MW or x-rays before or after MW irradiation, then plated onto medium containing TPA dissolved in acetone or just growth medium with acetone. Treatment with x-rays + TPA produced a significant increase in the transformation frequency compared with x-ray treatment alone. Treatment with MW + acetone produced no changes, but treatment with MW + TPA produced significant increases in the transformation frequency. "Thus, in the experiments reported here, microwaves appear to act as an initiator in a two-stage transformation assay" (Balcer-Kubiczek and Harrison 1989).

In a third experiment, there were no differences in the transformation frequencies between the sham-irradiated controls and the cells treated with just MW. MW exposure, then treatment with TPA, produced dose-rate dependent increases in transformations at 0.1, 1.0, and 4.4 W/kg. MW + x-rays + TPA greatly increased transformation rates. The authors conclude that their data seem to support the hypothesis that tumor promoters and modulated MW fields act at the cell membrane, including a synergistic action between TPA and MW (Balcer-Kubiczek and Harrison 1991).

Cleary, Liu, and Merchant (1990) exposed human glioma cells at 2450 MHz for 2 hours under isothermal conditions (37 ± 0.2°C). After exposure the cells were cultured, then evaluated for rates of DNA and RNA synthesis as indicated by cellular incorporation of radiolabeled nucleic acid precursors, ^{3}H-thymidine